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Bublot et al.

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- (54) **RECOMBINANT HVT VECTORS EXPRESSING ANTIGENS OF AVIAN PATHOGENS AND USES THEREOF**
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This patent is subject to a terminal disclaimer.
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- (51) **Int. Cl.**
A61K 39/12 (2006.01)
C07K 14/005 (2006.01)
C12N 15/86 (2006.01)
A61K 39/00 (2006.01)
- (52) **U.S. Cl.**
CPC *A61K 39/12* (2013.01); *C12N 15/86* (2013.01); *A61K 2039/5256* (2013.01); *A61K 2039/552* (2013.01); *A61K 2039/70* (2013.01); *C12N 2710/16334* (2013.01); *C12N 2710/16343* (2013.01); *C12N 2720/10034* (2013.01); *C12N 2760/18134* (2013.01); *C12N 2800/22* (2013.01)
- (58) **Field of Classification Search**
None
See application file for complete search history.

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Primary Examiner — Shanon A Foley**(74) Attorney, Agent, or Firm — Judy Jarecki-Black; Ruoying Chen; Merial, Inc.****(57) ABSTRACT**

The present invention provides recombinant herpesvirus of turkeys (HVT) vectors that contain and express antigens of avian pathogens, compositions comprising the recombinant HVT vectors, polyvalent vaccines comprising the recombinant HVT vectors and one or more wild type viruses or recombinant vectors. The present invention further provides methods of vaccination against a variety of avian pathogens and method of producing the recombinant HVT vectors.

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Figure 1

SEQ ID NO	Type	Gene
1	DNA	NDV-F VII ^d codon optimized DNA sequence
2	Protein	NDV-F protein sequence from codon-optimized VII ^d strain
3	DNA	NDV-F VII ^d wildtype sequence
4	Protein	NDV-F protein sequence from wildtype VII ^d strain
5	DNA	NDV-F Ca02 codon optimized DNA sequence
6	Protein	NDV-F protein sequence from codon-optimized Ca02 strain
7	DNA	IBDV DNA encoding VP2 protein
8	Protein	IBDV VP2 protein
9	DNA	SV40 promoter
10	DNA	CMV-IE promoter
11	DNA	SV40 polyA signal
12	DNA	Synthetic polyA signal
13	oligo	MB080 primer
14	oligo	MB081 primer
15	oligo	optF primer
16	oligo	VlloptF RP primer
17	oligo	SV40promoterF primer
18	DNA	Partial plasmid pHM103+Fopt DNA sequence (for vHVT114)
19	DNA	Partial plasmid pSB1 44 cds SV FCAopt (for vSB1-009)
20	DNA	Partial plasmid pHVT US2 SV- Fopt-synPA (for vHVT306)
21	DNA	Partial plasmid pCD046+NDV-F wt (for vHVT110)
22	DNA	Partial plasmid pHM103+NDV-F wt (for vHVT111)
23	DNA	Partial plasmid pHM103 + NDV-F CA02 (for vHVT116)
24	DNA	Partial plasmid HVTIG2 SV Fwt SbfI sequence (for vHVT301)
25	DNA	Partial plasmid pHVTUS10 cds F opt plasmid (for vHVT302)
26	DNA	Partial plasmid pHVTUS10 cds F CA02 opt sequence (for vHVT303)
27	DNA	Partial plasmid HVT IG2 SVFopt syn tail sequence (for vHVT304)
28	DNA	Partial plasmid pHVT US2 SV-FCA02 opt-synPA (for vHVT307)
29	DNA	Partial plasmid pCD046+NDV-F VII YZCQ sequence (vHVT112)
30	DNA	Partial plasmid pCD046+NDV Texas F sequence (for vHVT113)
31	DNA	Partial plasmid pHM119 sequence (for vHVT039)
32	DNA	NDV-F Wtnm-Texas wildtype DNA sequence
33	protein	NDV-F protein from Wtnm-Texas wildtype
34	DNA	NDV-F YZCQ wildtype DNA sequence
35	protein	NDV-F protein from wildtype YZCQ strain
36	DNA	NDV-F Texas wildtype DNA sequence
37	protein	NDV-F protein from wildtype Texas strain
38	DNA	MDV gB promoter

Figure 1 (continued)

SEQ ID NO	Type	Gene
39	DNA	Partial plasmid HVT SORF3-US2 gpVar-Ewtsyn sequence (for vHVT202)
40	DNA	Partial plasmid SB1US2 gpVIIdwtsyn sequence (for vSB1-010)
41	DNA	IBDV DNA encoding VP2 protein of IBDV E strain
42	protein	IBDV VP2 protein of IBDV E strain
43	DNA	Guinea pig CMV promoter
44	oligo	primer HM101
45	oligo	Primer HM102
46	oligo	primer F-ATG
47	oligo	Primer F-STOP

Figure 2

Genomic Structure of HVT, ORFs of the *BamHI* fragment,
and Insertion/Replacement Locations
(GenBank accession number for HVT FC126 sequence: AF291866.1)

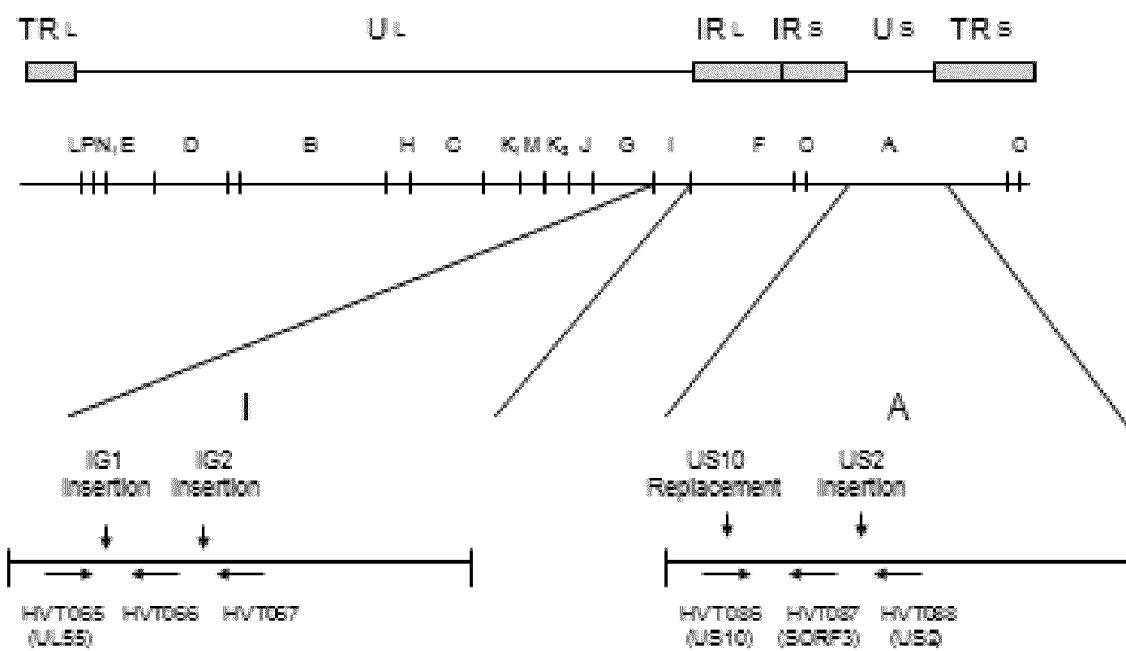


Figure 3

Plasmid map of pHM103 containing codon-optimized NDV-F gene

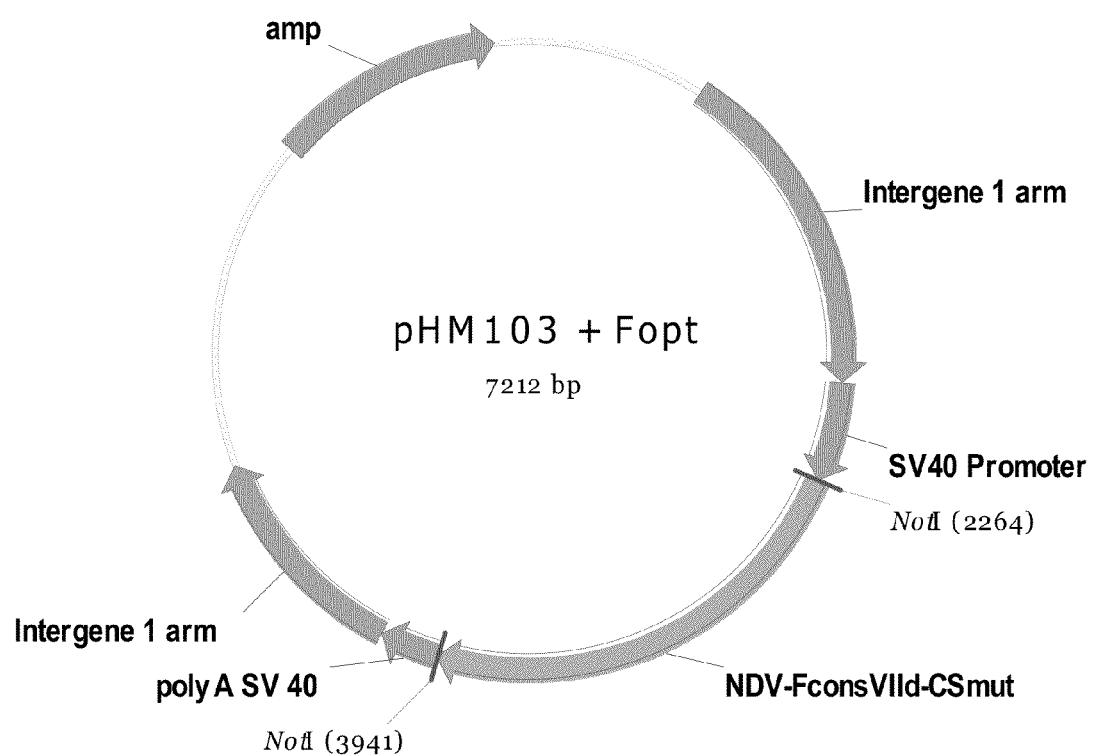
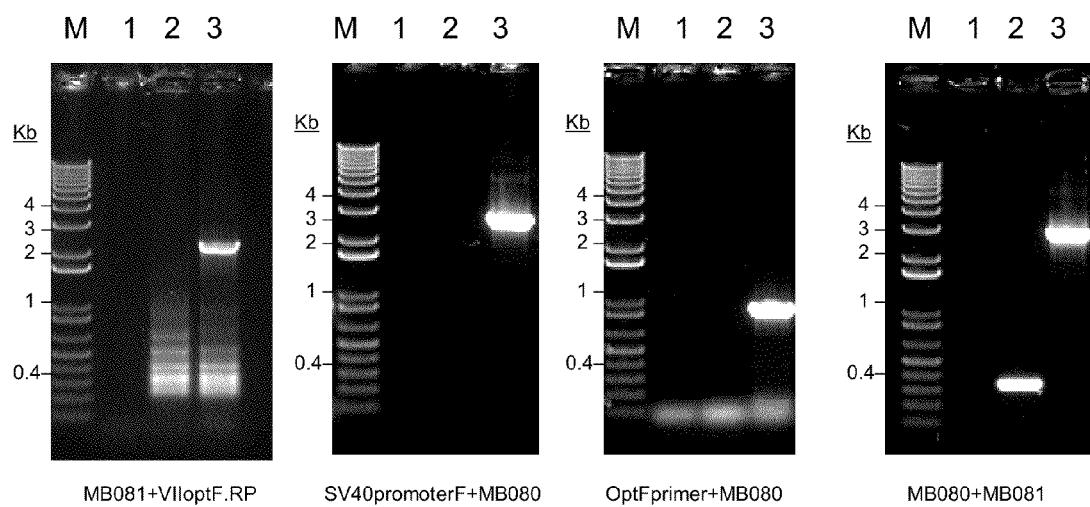


Figure 4

vHVT114 identity PCR



Lane 1: no template

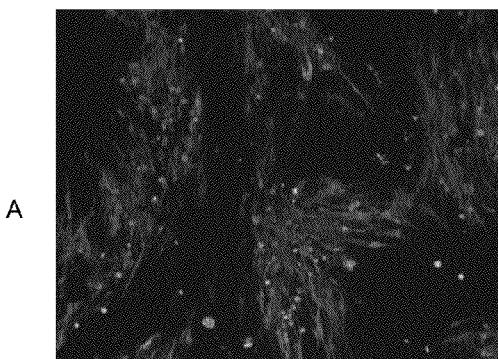
Lane 2: FC126 cl2

Lane 3: vHVT114

Figure 5

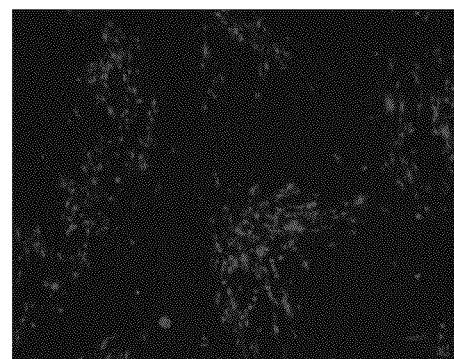
Dual Immunofluorescent Assay

FIG.5A1



A

FIG.5A2



B

FIG.5B1

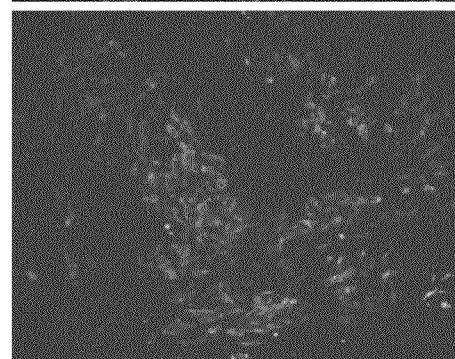
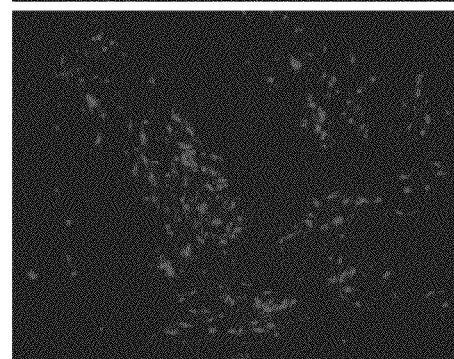


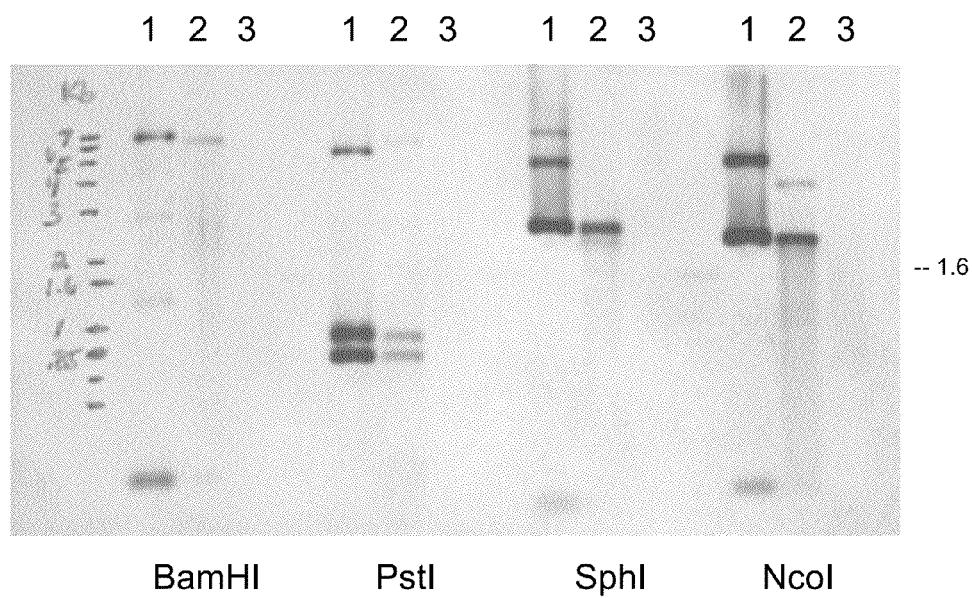
FIG.5B2



Panel A is from the pre-MSV passage
Panel B is from the pre-MSV+12 passage

Figure 6

Southern blot using the NDV-F probe



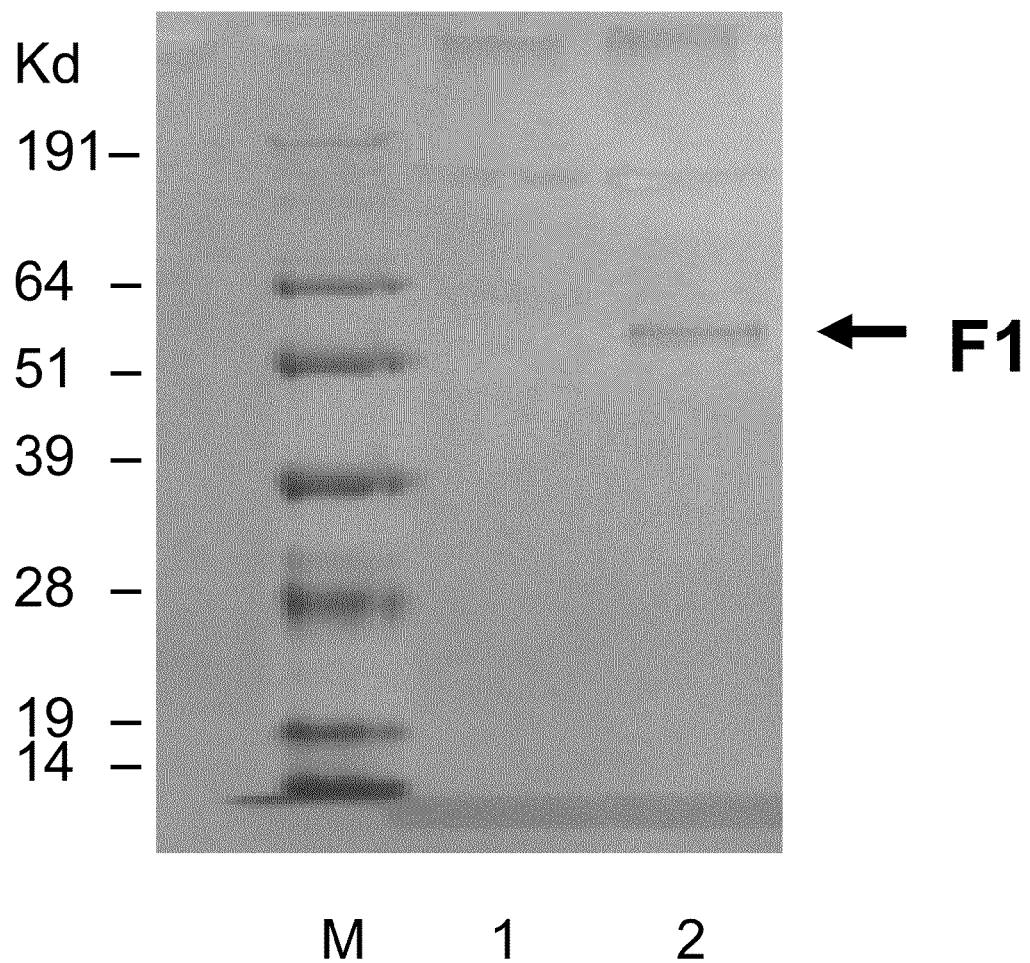
1 = pHM103+Fopt donor

2 = vHVT114

3 = FC126 cl2

Figure 7

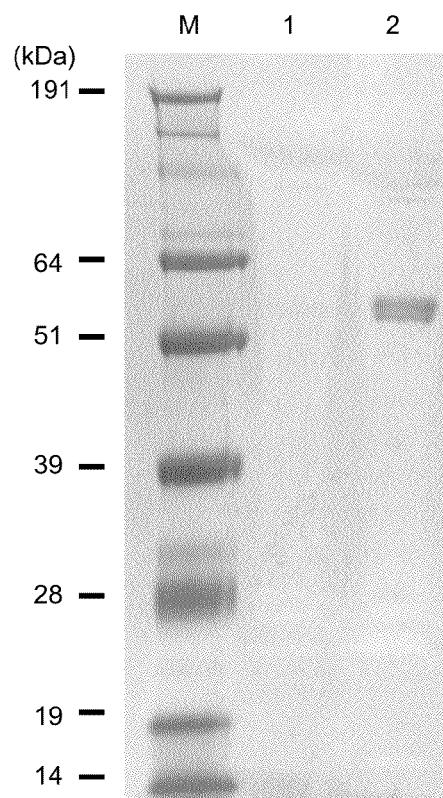
Immunoprecipitation and Western Blot of vHVT114



Lane M: Pre-Stained Standard (SeeBlue, Invitrogen);
Lane 1: CEF;
Lane 2: vHVT114.

Figure 8

Western blot analysis of immunoprecipitated sample from vHVT306 infected cells



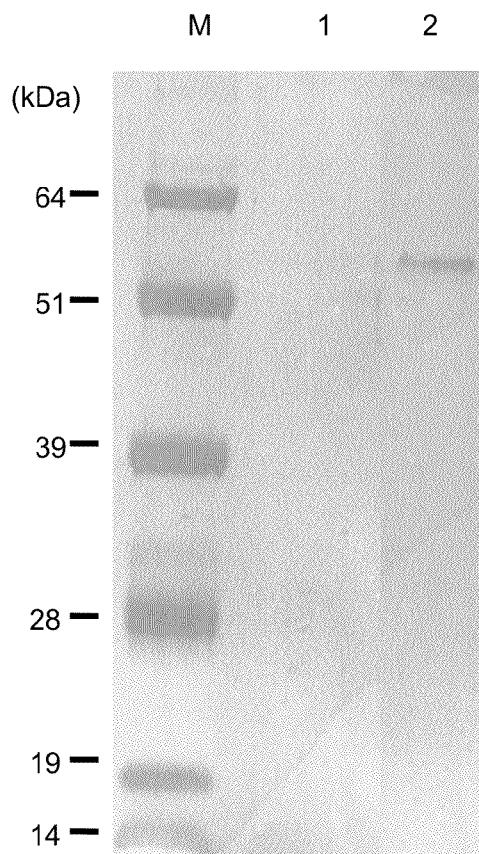
Lane M: pre-stained protein standard (Invitrogen, SeeBlue)

Lane 1: uninfected CEF

Lane 2: vHVT306

Figure 9

Western blot analysis of immunoprecipitated sample
from vSB1-009 infected cells



Lane M: pre-stained protein standard (Invitrogen, SeeBlue)

Lane 1: uninfected CEF

Lane 2: vSB1-009 pre-MSV stock

Figure 10

Challenge study of vHVT304 and vHVT114 against NDV ZJ1 and CA02

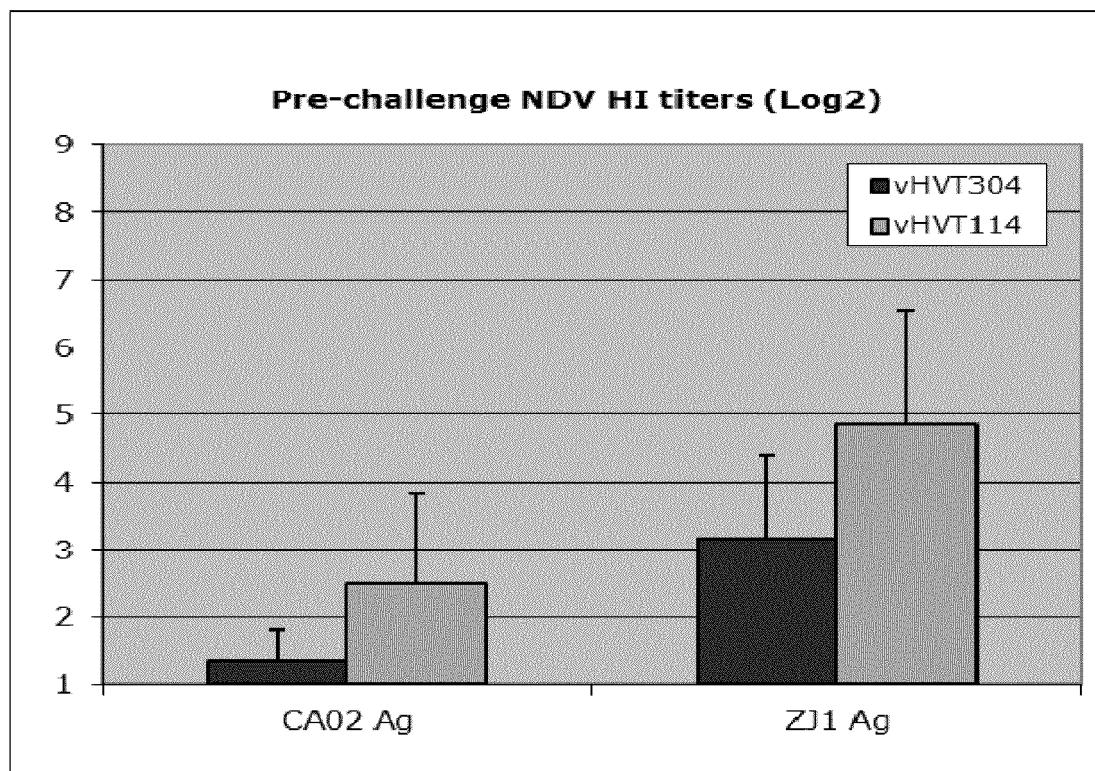


FIG. 11A

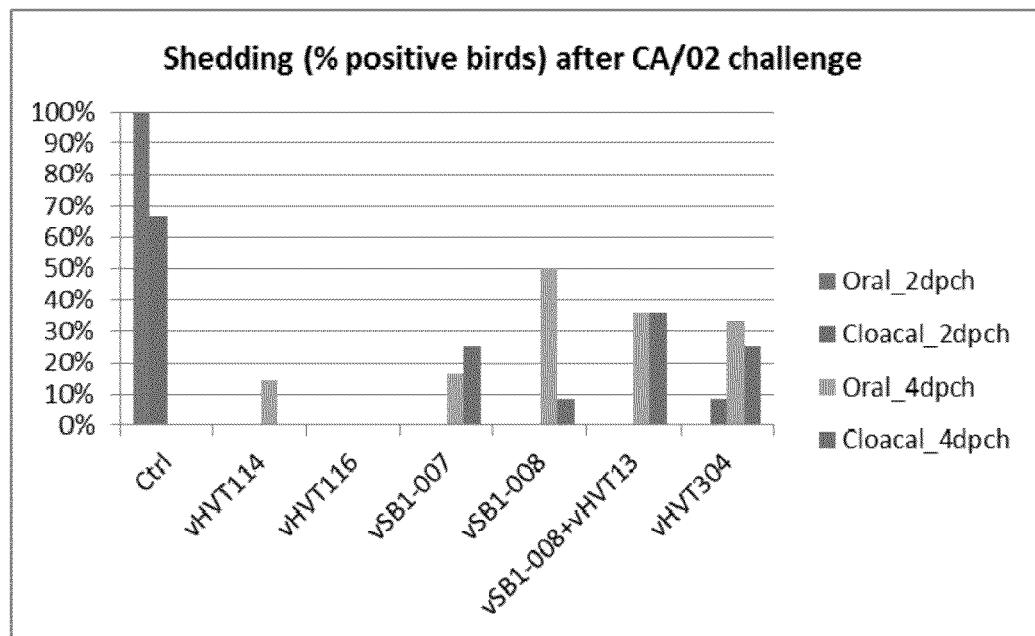


FIG. 11B

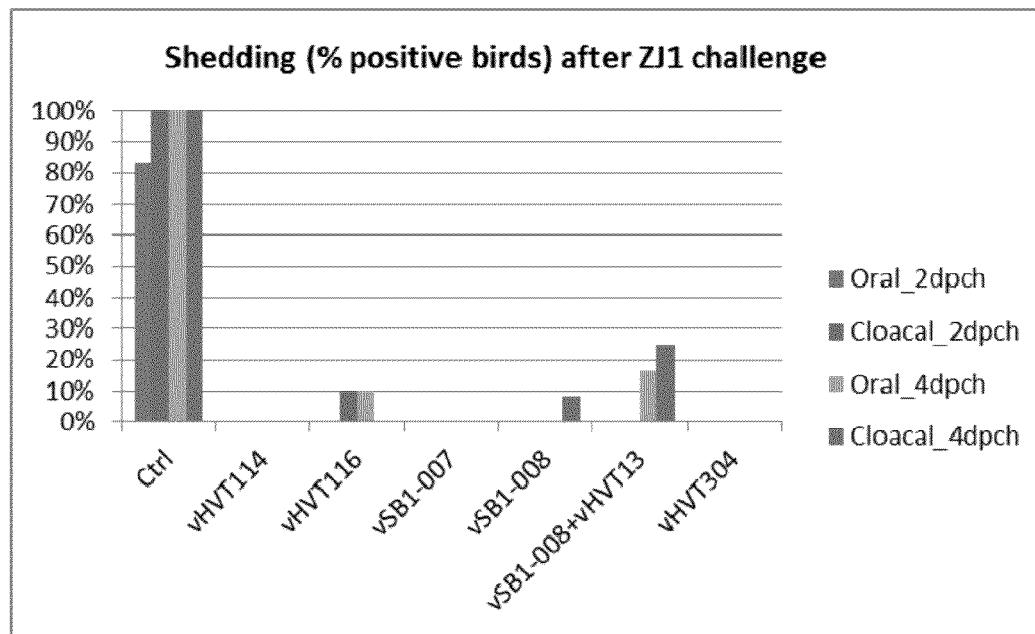


FIG. 12A

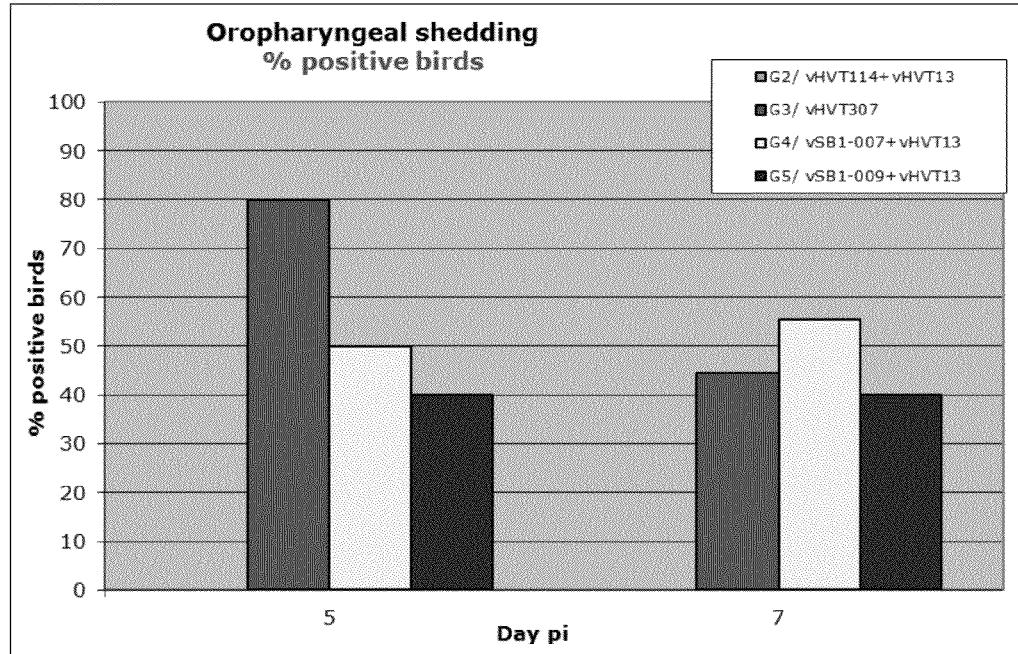


FIG. 12B

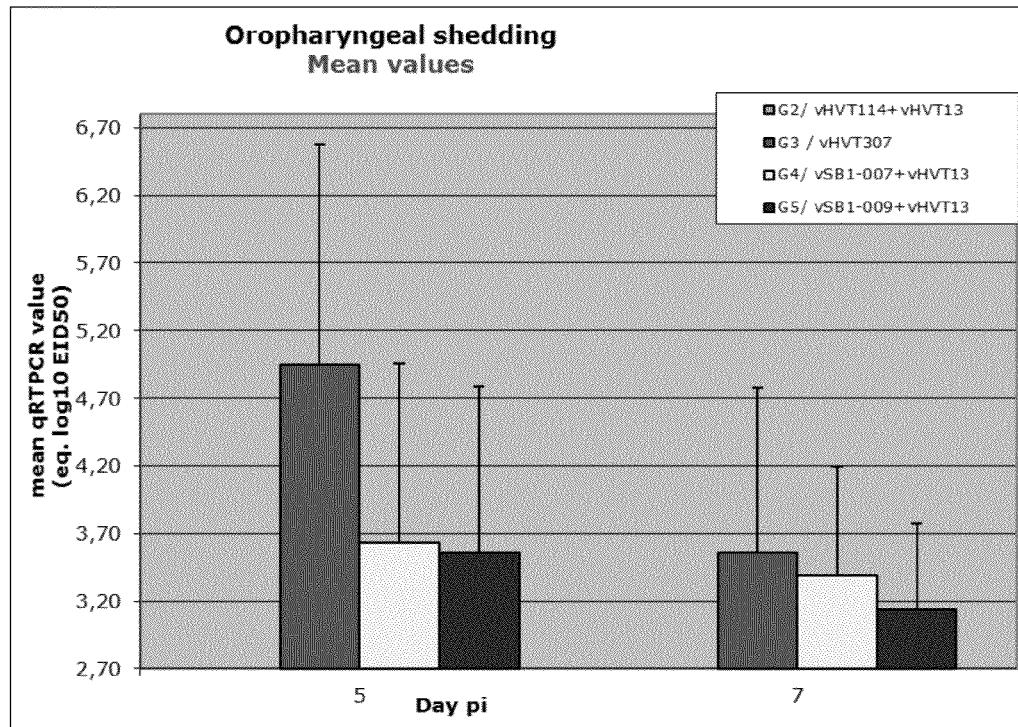


Figure 13

Protein sequence alignment of NDV-F

SEQ ID NO:2	1	50
SEQ ID NO:33	(1) MGSKPSTRI PAPLMLITRIMLILGCIRPTSSLDGRPLAAAGIVVTGDKAV	
SEQ ID NO:35	(1) MGSRSSTRIPVPLMLIIRTALTLS CIRLTSSLDGRPLAAAGIVVTGDKAV	
SEQ ID NO:37	(1) MGSKPSTRI PAPLMLITRIMLILDCIRPTSSLDGRPLAAAGIVVTGDKAV	
SEQ ID NO:4	(1) MGSKPSTRI PAPLMLITRIMLILGCIRPTSSLDGRPLAAAGIVVTGDKAV	
SEQ ID NO:6	(1) MGSKPSTWISVTIMLITRTMLILSCICPTSSLDGRPLAAAGIVVTGDKAV	
SEQ ID NO:2	51	100
SEQ ID NO:33	(51) NVYTSSQTGSIIIVKLLPNMPRDKEACAKAPLEAYNRTLTTLLTPLGDSIR	
SEQ ID NO:35	(51) NIYTSSQTGSIIIVKLLPNMPKDKEVCACAKAPLEAYNRTLTTLLTPLGDSIR	
SEQ ID NO:37	(51) NVYTSSQTGSIIIVKLLPNMPKDKEACAKDPLEAYNRTLTTLLTPLGDSIR	
SEQ ID NO:4	(51) NVYTSSQTGSIIIVKLLPNMPRDKEACAKAPLEAYNRTLTTLLTPLGDSIR	
SEQ ID NO:6	(51) NIYTSSQTGSIIIVKLLPNMPKDKEACAKAPLEAYNRTLTTLLTPLGDSIR	
SEQ ID NO:2	101	150
SEQ ID NO:33	(101) KIQGSVSTSGGGKQGRLIGAVIGSVALGVATAAQITAALIQANQNAAN	
SEQ ID NO:35	(101) RIOESVTTSGGRRQRFIGAIIGSVALGVATAAQITAASALIQANQNAAN	
SEQ ID NO:37	(101) RIOESVTTSGGGKQGRLIGAVIGSVALGVATAAQITAASALIQANQNAAN	
SEQ ID NO:4	(101) KIQGSVSTSGGGKQGRLIGAVIGSVALGVATAAQITAALIQANQNAAN	
SEQ ID NO:6	(101) KIQGSVSTSGGGKQGRLIGAVIGSVALGVATAAQITAALIQANQNAAN	
SEQ ID NO:2	151	200
SEQ ID NO:33	(151) ILRLKESIAATNEAVHEVT DGLSQLSVAVGKMQQFVN DQFNNTARELCI	
SEQ ID NO:35	(151) ILRLKESIAATNEAVHEVT DGLSQLAVAVGKMQQFVN DQFNNTARELCI	
SEQ ID NO:37	(151) ILRLKESIAATNEAVHEVT DGLSQLAVAVGKMQQFVN DQFNNTARELCI	
SEQ ID NO:4	(151) ILRLKESIAATNEAVHEVT DGLSQLSVAVGKMQQFVN DQFNNTARELCI	
SEQ ID NO:6	(151) ILRLKESIAATNEAVHEVT NGLSQLAVAVGKMQQFVN DQFNNTARELCI	
SEQ ID NO:2	201	250
SEQ ID NO:33	(201) KITQQVGVELNLYLTTELTTVFGPQITS PALTQLTIQALYNLAGGNMDYLL	
SEQ ID NO:35	(201) KIAQQVGVELNLYLTTELTTVFGPQITS PALTQLTIQALYNLAGGNMDYLL	
SEQ ID NO:37	(201) KIAQQVGVELNLYLTTELTTVFGPQITS PALTQLTIQALYNLAGGNMDYLL	
SEQ ID NO:4	(201) KITQQVGVELNLYLTTELTTVFGPQITS PALTQLTIQALYNLAGGNMDYLL	
SEQ ID NO:6	(201) KIAQQVGVELNLYLTTELTTVFGPQITS PALTQLTIQALYNLAGGNMDYLL	
SEQ ID NO:2	251	300
SEQ ID NO:33	(251) TKLGIGNNQLSSSLIGSGLITGYPILYDSQTOLLGIQVNLP S VGNLN NMRA	
SEQ ID NO:35	(251) TKLGVGNNQLSSSLIGSGLITGNPILYDSQTOLLGIQVNLP S VGNLN NMRA	
SEQ ID NO:37	(251) TKLGVGNNQLSSSLIGSGLITGNPILYDSQTOLLGIQVNLP S VGNLN NMRA	
SEQ ID NO:4	(251) TKLGIGNNQLSSSLIGSGLITGYPILYDSQTOLLGIQVNLP S VGNLN NMRA	
SEQ ID NO:6	(251) TKLGVGNNQLSSSLIGSGLITGNPILYDSQTOLLGIQVNLP S VGNLN NMRA	

Figure 13 (continued)

SEQ ID NO:2 (301)	301	350
SEQ ID NO:33 (301)	TYLETLSVTTKG Y ASALVPKVVTQVGSVIEELDTSYCIESDLDLYCTRI	
SEQ ID NO:35 (301)	TYLETLSVTTKG F ASALVPKVVTQVGSVIEELDTSYCIGTDLDLYCTRI	
SEQ ID NO:37 (301)	TYLETLSVSTA K G Y ASALVPKVVTQVGSVIEELDTSYCIESDLDLYCTRI	
SEQ ID NO:4 (301)	TYLETLSVTTKG Y ASALVPKVVTQVGSVIEELDTSYCIESDLDLYCTRI	
SEQ ID NO:6 (301)	TYLETLSVTTKG F ASALVPKVVTQVGSVIEELDTSYCIESDIDLYCTRV	
SEQ ID NO:2 (351)	351	400
SEQ ID NO:33 (351)	VTFPMSPGIYSCLSGNTSACMYSKTEGALTPYMALKG S VIANCKITT CR	
SEQ ID NO:35 (351)	VTFPMSPGIYSCLSGNTSACMYSKTEGALTPYMALKG S VIANCKLTT CR	
SEQ ID NO:37 (351)	VTFPMSPGIYSCLSGNTSACMYSKTEGALTPYMALKG S VIANCKITT CR	
SEQ ID NO:4 (351)	VTFPMSPGIYSCLSGNTSACMYSKTEGALTPYMALKG S VIANCKITT CR	
SEQ ID NO:6 (351)	VTFPMSPGIYSCLSGNTSACMYSKTEGALTPYMALKG S VIANCKMTT CR	
SEQ ID NO:2 (401)	401	450
SEQ ID NO:33 (401)	C T DPPGIISQNYGEAVSLIDRHSCNVLSLDGITLRLSGEFDATYQKNISI	
SEQ ID NO:35 (401)	CADPPGIISQNYGEAVSLIDRHSCNVLSLDGITLRLSGEFDATYQKNISI	
SEQ ID NO:37 (401)	C T DPPGIISQNYGEAVSLIDRHSCNVLSLDGITLRLSGEFDATYQKNISI	
SEQ ID NO:4 (401)	C T DPPGIISQNYGEAVSLIDRHSCNVLSLDGITLRLSGEFDATYQKNISI	
SEQ ID NO:6 (401)	CADPPGIISQNYGEAVSLIDKHSCSVLSLDGITLRLSGEFDATYQKNISI	
SEQ ID NO:2 (451)	451	500
SEQ ID NO:33 (451)	LDSQVITGNLDISTELGNVNNNSISNALDR I AESNSKLEKVNVR L STSA	
SEQ ID NO:35 (451)	LDSQVITGNLDISTELGNVNNNSISNALNK E ESNSKLDKVNV K LTSTSA	
SEQ ID NO:37 (451)	LDSQVITGNLDISTELGNVNNNSISNALDKLAKSN S KLEKVNVR L STSA	
SEQ ID NO:4 (451)	LDSQVITGNLDISTELGNVNNNSISNALDR I AESNSKLEKVNVR L STSA	
SEQ ID NO:6 (451)	LDSQVITGNLDISTELGNVNNNSIS T LDK L AESNNKLNKVNV N LTSTSA	
SEQ ID NO:2 (501)	501	550
SEQ ID NO:33 (501)	LITYIVLTVISL V FGALS V LACYL M K Q A QQ K TLLWLGNNTLDQMRAT	
SEQ ID NO:35 (501)	LITYIVLTVISL V FG V LS V LACYL M K Q A QQ K TLLWLGNNTLDQMRAT	
SEQ ID NO:37 (501)	LITYIVLTVISL V FGALS G LT C YL M K Q A QQ K TLLWLGNNTLDQMRAT	
SEQ ID NO:4 (501)	LITYIVLTVISL V FGALS V LACYL M K Q A QQ K TLLWLGNNTLDQMRAT	
SEQ ID NO:6 (501)	LITYIVLAI V SLAF G VISL V LACYL M K Q R QQ K TLLWLGNNTLDQMRAT	
SEQ ID NO:2 (551)	551	
SEQ ID NO:33 (551)	TRA--	
SEQ ID NO:35 (551)	TKI--	
SEQ ID NO:37 (551)	TRI--	
SEQ ID NO:4 (551)	TRA--	
SEQ ID NO:6 (551)	TRT--	

Figure 13 (continued)

	SEQ ID NO:2	SEQ ID NO:33	SEQ ID NO:35	SEQ ID NO:37	SEQ ID NO:4	SEQ ID NO:6
SEQ ID NO:2		92%	93% 99%	98% 92% 92%	100% 92% 93% 98%	92% 91% 92% 91% 92%
SEQ ID NO:33						
SEQ ID NO:35						
SEQ ID NO:37						
SEQ ID NO:4						
SEQ ID NO:6						

Figure 13 (continued)

DNA sequence alignment of NDV-F genes

SEQ ID NO:1	1	50
SEQ ID NO:3	(1) ATGGGCAGCAAGCCCAGCACAAGAATCCCAGCCCCCTGATGCTGATCAC	
SEQ ID NO:32	(1) ATGGGCTCCAAACCTTCTACCAGGATCCCGACACCTCTGATGCTGATCAC	
SEQ ID NO:34	(1) ATGGGCTCCAGATCTTCTACCAGGATCCCGTACCTCTAATGCTGATCAT	
SEQ ID NO:36	(1) ATGGGCTCTAACCTTCTACCAGGATCCCGTACCTCTAATGCTGATCAT	
SEQ ID NO:5	(1) ATGGGCAGCAAGCCCAGCACCTGGATCAGCGTGACCCCTGATGCTGATCAC	
SEQ ID NO:1	51	100
SEQ ID NO:3	(51) CCGCATCATGCTGATCCTGGGCTGCATCAGACCCACAAGCTCCCTGGATG	
SEQ ID NO:32	(51) CCGGATTATGCTGATATTGGGCTGTATCCGTCGACAAGCTCTTGTACG	
SEQ ID NO:34	(51) CCGAACCGCGCTGACACTGAGCTGTATCCGTCGACAAGCTCTTGTACG	
SEQ ID NO:36	(51) CCGGATTATGCTGATATTGGACTGTATCCGTCGACAAGCTCTTGTACG	
SEQ ID NO:5	(51) CAGAACCATGCTGATCCTGAGCTGCATCTGCCCAACAAGCAGCTGGACG	
SEQ ID NO:1	101	150
SEQ ID NO:3	(101) GACGCCCCCTGGCCGCTGCCGGCATCGTGGTACCGGGCGACAAGGCCGTG	
SEQ ID NO:32	(101) GCAGGCCCTTGGCAGCTGCAGGAATTGTAGTAACAGGAGATAAGGCAGTC	
SEQ ID NO:34	(101) GCAGGCCCTTGGCAGCTGCAGGGATCGTGGTAACAGGAGATAAGGCAGTC	
SEQ ID NO:36	(101) GCAGGCCCTTGGCAGCTGCAGGAATTGTAGTAACAGGAGATAAGGCAGTC	
SEQ ID NO:5	(101) GCAGAACCCCTGGCCGCTGCCGGCATCGTGGTACCGGGCGACAAGGCCGTG	
SEQ ID NO:1	151	200
SEQ ID NO:3	(151) AACGTGTACACCGAGCAGCCAGACCCGGCAGCATCATCGTGAAGCTGCTGCC	
SEQ ID NO:32	(151) AATGTATACACTTCTCGTCTCAGACAGGGTCAATCATAGTCAAGTTGCTCCC	
SEQ ID NO:34	(151) AACATATACACCTCATCCCAGACAGGGTCAATCATAGTTAAGTTACTCCC	
SEQ ID NO:36	(151) AACATATACACCTCATCCCAGACAGGGTCAATCATAGTTAAGTTACTCCC	
SEQ ID NO:5	(151) AATGTATATACCTCGTCTCAGACAGGGTCAATCATAGTCAAGTTGCTCCC	
SEQ ID NO:5	(151) AACATCTACACCAGCAGCCAGACCCGGCAGCATCATCAAGCTGCTGCC	
SEQ ID NO:1	201	250
SEQ ID NO:3	(201) CAACATGCCAGAGACAAAGAGGCCCTGCCAAGGCCCTGGAAAGCCT	
SEQ ID NO:32	(201) GAATATGCCAGATAAGGAGGCCCTGCCAAAAGCCCCATTAGAGGCAT	
SEQ ID NO:34	(201) GAATATGCCAAGGACAAAGAGGTGTGTGCCAAAAGCCCCATTGGAGGCAT	
SEQ ID NO:36	(201) GAATATGCCAAGGACAAAGAGGTGTGTGCCAAAAGCCCCATTAGAGGCAT	
SEQ ID NO:5	(201) CAACATGCCAAGGACAAAGAGGCCCTGCCAAGGCCCTGGAAAGCCT	
SEQ ID NO:1	251	300
SEQ ID NO:3	(251) ACAACAGAACCTGACCACCTGCTGACCCCCCTGGCGACAGCATCAGA	
SEQ ID NO:32	(251) ATAACAGAACACTGACTACTTGTCTCACTCCTCTTGGCGACTCCATCCGC	
SEQ ID NO:34	(251) ACAACAGGACACTGACTACTTACTCACCCCCCTTGGTGAATTCTATCCGC	
SEQ ID NO:36	(251) ACAACAGGACACTGACTACTTACTCACCCCCCTTGGTGAATTCTATCCGC	
SEQ ID NO:5	(251) ATAACAGAACACTGACTACTTGTCTCACTCCTCTTGGCGAATCCATCCGC	
SEQ ID NO:5	(251) ACAACAGAACCTGACCACCTGCTGACCCCCCTGGCGACAGCATCAGA	

Figure 13 (continued)

	301	350
SEQ ID NO:1	(301) AAGATCCAGGGCTCCGTGAGCACAAAGCGGGGGAGGAAGCAGGGCAGACT	
SEQ ID NO:3	(301) AAGATCCAACGGTCTGTGTCACATCTGGAGGAGGCAAGCAAGGGCGCT	
SEQ ID NO:32	(301) AGGATACAACAGTCTGTGACTACTTCCGGAGGAAGGAGACAGAGACGCTT	
SEQ ID NO:34	(301) AGGATACAACAGTCTGTGACTACTTCCGGAGGAGGCAAGCAAGGGCGCT	
SEQ ID NO:36	(301) AAGATCCAACGGTCTGTGTCACGTCTGGAGGAGGCAAGCAAGGGCGCT	
SEQ ID NO:5	(301) AGAATCCAGGGCAGGCCACCACAAGCGGGGGAGGAAGCAGGGCAGACT	
	351	400
SEQ ID NO:1	(351) GATCGGGCCCGTGTGATCGGCAGCGTGGCCCTGGGAGTGGCTACAGCTGCC	
SEQ ID NO:3	(351) GATAAGGTGCTGTTATTGGCAGTGAGCTCTTGGGGTTGCAACAGCGGCAC	
SEQ ID NO:32	(351) TATAAGGTGCCATTATCGGCAGTGAGCTCTTGGGGTTGCGACAGCTGCAC	
SEQ ID NO:34	(351) GATAAGGTGCCATTATCGGCAGTGAGCTCTTGGGGTTGCGACAGCTGCAC	
SEQ ID NO:36	(351) GATAAGGTGCTGTTATTGGTAGTGAGCTCTTGGGGTTGCAACAGCGGCAC	
SEQ ID NO:5	(351) GGTGGGCCCTATCATCGGGAGCGTGGCCCTGGGCGTGGCACAGCTGCC	
	401	450
SEQ ID NO:1	(401) AGATTACCGCTGCAGCCGCCCTGATCCAGGCCAACAGAACGCCAAC	
SEQ ID NO:3	(401) AGATAACAGCAGCTCGGCCCTAAATACAAGCCAACCAGAACGCCAAC	
SEQ ID NO:32	(401) AGATAACAGCAGCTCGGCCCTGATACAAGCCAACCAGAACGCCAAC	
SEQ ID NO:34	(401) AGATAACAGCAGCTCGGCCCTGATACAAGCCAACCAGAACGCCAAC	
SEQ ID NO:36	(401) AAATAACAGCAGCTCGGCCCTAAATACAAGCCAACCAGAACGCCAAC	
SEQ ID NO:5	(401) AGATTACCGCTGCAGCCGCCCTGATTCAAGGCCAACAGAACGCCAAC	
	451	500
SEQ ID NO:1	(451) ATCCTGAGACTGAAAGAGAGCATTGCCGCCACCAACGAGGCCGTGCACGA	
SEQ ID NO:3	(451) ATCCTCCGGCTTAAGGAGAGCATTGCTGCAACCAATGAAAGCTGTGCATGA	
SEQ ID NO:32	(451) ATCCTCCGGCTTAAGGAGAGCATTGCTGCAACCAATGAAAGCTGTGCACGA	
SEQ ID NO:34	(451) ATCCTCCGGCTTAAGGAGAGCATTGCTGCAACCAATGAAAGCTGTGCACGA	
SEQ ID NO:36	(451) ATCCTCCGGCTTAAGGAGAGCATTGCTGCAACCAATGAAAGCTGTGCATGA	
SEQ ID NO:5	(451) ATCCTGAGACTGAAAGAGAGCATTGCCGCCACCAACGAGGCCGTGCACGA	
	501	550
SEQ ID NO:1	(501) AGTGACCGACGGCTGAGCCAGCTGTCCGTGGCGTGGCAAGATGCAGC	
SEQ ID NO:3	(501) AGTCACCGACGGATTATCACAACATCAGTGGCAGTTGGAAAGATGCAGC	
SEQ ID NO:32	(501) GGTCACTGACGGATTATCACAACATCAGTGGCAGTAGGAAAGATGCAAC	
SEQ ID NO:34	(501) GGTCACTGACGGATTATCACAACATCAGTGGCAGTAGGAAAGATGCAAC	
SEQ ID NO:36	(501) AGTCACCGACGGATTATCACAACATCAGTGGCAGTTGGAAAGATGCAGC	
SEQ ID NO:5	(501) AGTGACAAACGGACTGTCCCAGCTGGCTGCGCTGCGCAAGATGCAGC	
	551	600
SEQ ID NO:1	(551) AGTCGTGAACGACCAGTTCAACAAACACCGCCAGAGAGCTGGACTGCATC	
SEQ ID NO:3	(551) AGTTTGTCATGACCAGTTAAATAATACAGCGCAGAATTGGACTGTATA	
SEQ ID NO:32	(551) AGTTTGTCATGACCAGTTCAATAATACAGCGCAGAATTGGACTGTATA	
SEQ ID NO:34	(551) AGTTTGTCATGACCAGTTCAATAATACAGCGCAGAATTGGACTGTATA	
SEQ ID NO:36	(551) AGTTTGTCATGACCAGTTAAATAATACAGCGCAGAATTGGACTGTATA	
SEQ ID NO:5	(551) AGTCGTGAACAAACCGCCAGAGAGCTGGACTGCATC	

Figure 13 (continued)

SEQ ID NO:1 SEQ ID NO:3 SEQ ID NO:32 SEQ ID NO:34 SEQ ID NO:36 SEQ ID NO:5	(601) (601) (601) (601) (601) (601)	601 AAGATCACCCAGCAGGTGGGCTGGAGCTAACCTGTACCTGACCGAGCT AAAATCACACAACAGGTTGGTAGAACTCAACCTATACCTAACGTGAATT AAAATTGCACAGCAGGTGGTAGAACTCAACTTGACCTAACGTGAATT AAAATTGCACAGCAGGTGGTAGAACTCAACTTGACCTAACGTGAATT AAAATCACACAACAGGTTGGTAGAACTCAACCTATACCTAACGTGAATT AAGATGCCAGCAGGTGGGCTGGAGCTAACCTGTACCTGACCGAGCT	650
SEQ ID NO:1 SEQ ID NO:3 SEQ ID NO:32 SEQ ID NO:34 SEQ ID NO:36 SEQ ID NO:5	(651) (651) (651) (651) (651) (651)	651 GACCACAGTGGCGCCCCAGATCACAAAGCCCAGCCTGACACAGCTGA GACTACAGTATTGGGCCACAGATCACCTCCCTGCATTAACTCAGCTGA GACTACAGTATTGGGCCACAAATCACTTCCCTGCCTTAACTCAGCTGA GACTACAGTATTGGGCCACAGATCACCTCCCTGCATTAACTCAGCTGA GACTACAGTATTGGGCCACAGATCACCTCCCTGCATTAACTCAGCTGA GACCACAGTGGCGCCCCAGATCACAAAGCCCGCTGTACCCAGCTGA	700
SEQ ID NO:1 SEQ ID NO:3 SEQ ID NO:32 SEQ ID NO:34 SEQ ID NO:36 SEQ ID NO:5	(701) (701) (701) (701) (701) (701)	701 CCATCCAGGCCCTGTACAACCTGGCTGGCGAACATGGACTATCTGCTG CCATCCAGGCCACTTTATAATTAGCTGGGGCAATATGGATTACTTGCTG CTATCCAAGCGCTTACAATCTAGCTGGGGTAATATGGATTACTTGCTG CTATCCAAGCGCTTACAATCTAGCTGGGGTAATATGGATTACTTGCTG CCATCCAGGCCACTTTATAATTAGCTGGGGCAATATGGATTACTTGCTG CAATCCAGGCCCTGTACAACCTGGCTGGCGAACATGGACTATCTGCTG	750
SEQ ID NO:1 SEQ ID NO:3 SEQ ID NO:32 SEQ ID NO:34 SEQ ID NO:36 SEQ ID NO:5	(751) (751) (751) (751) (751) (751)	751 ACAAAGCTGGGAATCGGCAACAAACCAGCTGTCCAGCCTGATCGGAAGCGG ACTAAGTTAGGTATAGGAACAATCAACTCAGCTCGTTAATTGGTAGCGG ACTAAGTTAGGTAGGGACAACCAACTCAGCTCATTAATTGGTAGCGG ACTAAGTTAGGTAGGGACAACCAACTCAGCTCATTAATTGGTAGCGG ACTAAGTTAGGTATAGGAACAATCAACTCAGCTCATTAATTGGCAGCGG ACTAAGCTGGGAATGGCAACAAACCAGCTGTCCAGCCTGATCGGTCCGG	800
SEQ ID NO:1 SEQ ID NO:3 SEQ ID NO:32 SEQ ID NO:34 SEQ ID NO:36 SEQ ID NO:5	(801) (801) (801) (801) (801) (801)	801 CCTGATCACCGGCTACCCATCCTGTACGACAGCCAGACACAGCTGCTGG CCTGATCACTGGTACCCATACTGTATGACTCACAGACTCAACTCTTGG CTTGATCACCGGCAACCTATTCTGTACGACTCACAGACTCAGATCTTGG CTTGATCACCGGCAACCTATTCTGTACGACTCACAGACTCAGATCTTGG CCTGATCACTGGTACCCATTGTATGACTCACAGACTCAACTCTTGG GCTGATCACAGGCAACCCATCCTGTACGACAGCCAGACACAGCTGCTGG	850
SEQ ID NO:1 SEQ ID NO:3 SEQ ID NO:32 SEQ ID NO:34 SEQ ID NO:36 SEQ ID NO:5	(851) (851) (851) (851) (851) (851)	851 GCATCCAGGTGAACCTGCCAGCGTGGCAACCTGAACAAACATGCCGCC GCATACAAGTGAATTACCCCTCAGTCGGGAACCTAAATAATATGCCGTGCC GTATACAGGTAACTTTGCCTTCAGTTGGGAACCTGAATAATATGCCGTGCC GTATACAGGTAACTTTGCCTTCAGTTGGGAACCTGAATAATATGCCGTGCC GCATACAAGTGAATTGCCTTCAGTCGGGAACCTAAATAATATGCCGTGCC GCATCCAGATCAACCTGCCATCCGTGGGAAGCCTGAACAAACATGAGAGCC	900

Figure 13 (continued)

	901	950
SEQ ID NO:1	(901) ACCTACCTGGAAACCTGAGCGTGTCCACCACCAAGGGTACGCCAGCGC	
SEQ ID NO:3	(901) ACCTATTGGAGACCTTATCTGAAGTACAACCAAGGATATGCCTCAGC	
SEQ ID NO:32	(901) ACCTACCTGGAGACCTTATCTGAAGCACAACCAAGGGATTGCCTCAGC	
SEQ ID NO:34	(901) ACCTACCTGGAGACCTTATCTGAAGCACAACCAAGGGATTGCCTCAGC	
SEQ ID NO:36	(901) ACCTATTAGAGACCTTATCTGAAGTACAGCAAAGGATATGCCTCAGC	
SEQ ID NO:5	(901) ACCTACCTGGAAACCTGAGCGTGTCCACCACCAAGGGTTGCCAGCGC	
	951	1000
SEQ ID NO:1	(951) CCTGGTGCCCAGGTGGTGACACAGGTGGCAGCGTGTGAGGAACTGG	
SEQ ID NO:3	(951) ACTTGTCCCAGGTGGTGACACAGGTGGTGTGAGGAACTGG	
SEQ ID NO:32	(951) ACTTGTCCCAGGTGGTGACACAGGTGGTGTGAGGAACTGG	
SEQ ID NO:34	(951) ACTTGTCCCAGGTGGTGACACAGGTGGTGTGAGGAACTGG	
SEQ ID NO:36	(951) ACTTGTCCCAGGTGGTGACACAGGTGGTGTGAGGAACTGG	
SEQ ID NO:5	(951) CCTGGTGCCCAGGTGGTGACACAGGTGGCAGCGTGTGAGGAACTGG	
	1001	1050
SEQ ID NO:1	(1001) ACACCAAGCTACTGCATCGAGAGCGACCTGGACCTGTACTGCACCAAGAATC	
SEQ ID NO:3	(1001) ACACCTCATACTGTATAGAGTCGATCTGGATTATATTGTACTAGAATA	
SEQ ID NO:32	(1001) ACACCTCATACTGTATAGGGACCGACTTGGATTATACTGTACAAAGAATA	
SEQ ID NO:34	(1001) ACACCTCATACTGTATAGGGACCGACTTGGATTATACTGTACAAAGAATA	
SEQ ID NO:36	(1001) ACACCTCATACTGTATAGACTCCGATCTGGATTATATTGTACTAGAATA	
SEQ ID NO:5	(1001) ACACCAAGCTACTGCATCGAGAGCGACATCGACCTGTACTGCACCAAGGT	
	1051	1100
SEQ ID NO:1	(1051) GTGACCTTCCCATTGAGCCCCGGCATCTACAGCTGCCGTGAGCGGCAACAC	
SEQ ID NO:3	(1051) GTGACATTCCCCATGCTCTGGTATTCTGTCTGAGCGGCAACAC	
SEQ ID NO:32	(1051) GTGACATTCCCATTGCTCTGGTATTCTGTCTGAGCGGTAATAC	
SEQ ID NO:34	(1051) GTGACATTCCCATTGCTCTGGTATTCTGTCTGAGCGGTAATAC	
SEQ ID NO:36	(1051) GTGACATTCCCATTGCTCTGGTATTCTGTCTGAGCGGCAACAC	
SEQ ID NO:5	(1051) GTGACCTTCCCATTGAGCCCCGGCATCTACAGCTGCCGTGAGCGGCAACAC	
	1101	1150
SEQ ID NO:1	(1101) CAGCGCCCTGCATGTACAGCAAGACCGAAGGGCGACTGACAACACCTACA	
SEQ ID NO:3	(1101) ATCAGCTTGCATGTATTCAAAGACTGAAGGGCGACTCACTACGCCGTATA	
SEQ ID NO:32	(1101) ATCGGCTTGCATGTATTCAAAGACTGAAGGGCGACTTACTACGCCATATA	
SEQ ID NO:34	(1101) ATCGGCTTGCATGTATTCAAAGACTGAAGGGCGACTTACTACGCCATATA	
SEQ ID NO:36	(1101) ATCAGCTTGCATGTATTCAAAGACTGAAGGGCGACTCACTACGCCGTATA	
SEQ ID NO:5	(1101) CAGCGCCCTGCATGTACAGCAAGACCGAAGGGAGCAGTACAACACCTACA	
	1151	1200
SEQ ID NO:1	(1151) TGGCCCTGAAGGGAAAGCGTGTGACGCCAACTGCAAGATCACCACTGCAGA	
SEQ ID NO:3	(1151) TGGCCCTTAAAGGCTCAGTTATTGCCAATTGCAAGCTGACAACATGTAGA	
SEQ ID NO:32	(1151) TGGCTCTCAAAGGCTCAGTTATTGCCAATTGCAAGCTGACAACATGTAGA	
SEQ ID NO:34	(1151) TGGCTCTCAAAGGCTCAGTTATTGCCAATTGCAAGCTGACAACATGTAGA	
SEQ ID NO:36	(1151) TGGCCCTTAAAGGCTCAGTTATTGCCAATTGTAAGATAACAACATGTAGA	
SEQ ID NO:5	(1151) TGGCCCTGAAGGGAAAGCGTGTGACGCCAACTGCAAGATGACCACCTGCAGA	

Figure 13 (continued)

SEQ ID NO:1 (1201)	1201	SEQ ID NO:1 (1250)	1250
TGCACCGACCCCCCAGGCATCATCAGCCAGAACTACGGCGAGGCCGTGAG		TGTACAGACCCCTCCTGGTATCATATCGCAAATTATGGAGAAGCTGTATC	
SEQ ID NO:3 (1201)		TGTGCAGATCCCCCAGGTATCATATCGCAAATTATGCAGAAGCTGTGTC	
SEQ ID NO:32 (1201)		TGTGCAGATCCCCCAGGTATCATATCGCAAATTATGGAGAAGCTGTGTC	
SEQ ID NO:34 (1201)		TGTACAGACCCCTCCTGGTATCATATCGCAAATTATGGAGAAGCTGTATC	
SEQ ID NO:36 (1201)		TGCACCGACCCCCCAGGCATCATCAGCCAGAACTACGGCGAGGCCGTGAG	
SEQ ID NO:5 (1201)			
1251			
SEQ ID NO:1 (1251)		CCTGATCGATCGCCATTCTGTAAACGTGCTGTCCCTGGACGGCATCACAC	
SEQ ID NO:3 (1251)		CCTGATAGATAGACATTGTGCAATGTCTTATCATTAGACGGGATAACTC	
SEQ ID NO:32 (1251)		CTTAATAGATAGGCACTCATGAAACGTCTTATCCTTAGACGGGATAACTC	
SEQ ID NO:34 (1251)		CTTAATAGATAGGCACTCATGAAACGTCTTATCCTTAGACGGGATAACTC	
SEQ ID NO:36 (1251)		CCTGATAGATAGACATTGTGCAATGTCTTATCATTAGACGGGATAACTC	
SEQ ID NO:5 (1251)		CCTGATCGACAAACATTCTGTAGCGTGCTGTCCCTGGATGGCATCACAC	
1301			
SEQ ID NO:1 (1301)		TGAGACTGAGCGGGCAGTTCGATGCCACCTACCAGAAGAACATCAGCATT	
SEQ ID NO:3 (1301)		TAAGGCTCAGTGGGAATTGATGCAACTTATCAAAGAACATCTCAATA	
SEQ ID NO:32 (1301)		TGAGGCTCAGTGGGAATTGATGCAACCTATCAAAGAACATCTCTATA	
SEQ ID NO:34 (1301)		TGAGGCTCAGTGGGAATTGATGCAACCTATCAAAGAACATCTCTATA	
SEQ ID NO:36 (1301)		TGAGGCTCAGTGGGAATTGATGCAACTTATCAAAGAACATCTCAATA	
SEQ ID NO:5 (1301)		TGAGACTGAGCGGGCAGTTCGACGCCACCTACCAGAAGAACATCAGCATT	
1351			
SEQ ID NO:1 (1351)		CTGGACAGCCAGGTGATCGTGACCGGGCAACCTGGACATCAGCACCGAGCT	
SEQ ID NO:3 (1351)		CTAGATTCTCAAGTCATCGTGACAGGCAATCTTGATATATCAACTGAACT	
SEQ ID NO:32 (1351)		CTAGATTCTCAAGTTAGTGACAGGCAATCTTGATATATCAACTGAGCT	
SEQ ID NO:34 (1351)		CTAGATTCTCAAGTTAGTGACAGGCAATCTTGATATATCAACTGAGCT	
SEQ ID NO:36 (1351)		CTAGATTCTCAAGTCATCGTGACAGGCAATCTTGATATATCAACTGAACT	
SEQ ID NO:5 (1351)		CTGGACAGCCAGGTGATCGTGACCGGGCAACCTGGACATCAGCACCGAGCT	
1401			
SEQ ID NO:1 (1401)		GGGCAACGTGAATAACAGCATCAGCAACGCCCTGGACAGACTGGCCGAGA	
SEQ ID NO:3 (1401)		TGGAAACGTCAACAATTCAATCAGCAATGCCCTGGATAGGTTGCCAGAAA	
SEQ ID NO:32 (1401)		TGGGAATGTCAACAATTCAATAAGTAATGCCCTGAATAAGTTAGAGGAAA	
SEQ ID NO:34 (1401)		TGGGAATGTCAACAATTCAATAAGTAATGCCCTGAATAAGTTAGAGGAAA	
SEQ ID NO:36 (1401)		TGGAAACGTCAACAATTCAATCAGCAATGCCCTGGATAAGTTGCCAAAAAA	
SEQ ID NO:5 (1401)		GGGCAACGTGAACAAACAGCATCAGCAGCACCCCTGGACAAGCTGGCCAGT	
1451			
SEQ ID NO:1 (1451)		GCAACAGCAAGCTGGAAAAAGTGAACGTGCGCCTGACATCCACTTCCGCT	
SEQ ID NO:3 (1451)		GCAACAGCAAGCTAGAAAAAGTCAATGTCAAGACTAACAGCACATCTGCT	
SEQ ID NO:32 (1451)		GCAACAGCAAAACTAGACAAAGTCAATGTCAAACACTGACCAAGCACATCTGCT	
SEQ ID NO:34 (1451)		GCAACAGCAAAACTAGACAAAGTCAATGTCAAACACTGACCAAGCACATCTGCT	
SEQ ID NO:36 (1451)		GCAACAGCAAGCTAGAAAAAGTCAATGTCAAGACTAACAGCACATCCGCT	
SEQ ID NO:5 (1451)		CCAACAACAAGCTGAACAAAGTGAACGTGAAACCTGACCAAGCACAGCGCC	

Figure 13 (continued)

	1501		1550
SEQ ID NO:1 (1501)	CTGATCACCTACATCGCTGACCGTGTACAGCCTGGTGTTCGGCGCCCT		
SEQ ID NO:3 (1501)	CTCATTACCTATATTGTTCAACTGTCAATTCTCTAGTTTCGGTGCACT		
SEQ ID NO:32 (1501)	CTCATTACCTACATCGTTAACGTCAATCTCTTGTGTTGGTGTACT		
SEQ ID NO:34 (1501)	CTCATTACCTACATCGTTAACGTCAATCTCTTGTGTTGGTGTACT		
SEQ ID NO:36 (1501)	CTCATTACCTATATTGTTCTGACTGTCAATTCTCTAGTTTCGGTGCACT		
SEQ ID NO:5 (1501)	CTGATCACCTACATCGCTGGCATCGTGTCCCTGGCCTTCGGCGTGAT		
	1551		1600
SEQ ID NO:1 (1551)	GAGCCTGGTGTGGCTGCTACCTGTACAAGCAGAAGGCCAGCAGA		
SEQ ID NO:3 (1551)	TAGTCTGGTGTAGCGTGTACCTGTACAACACAGAAGGCACAACAAA		
SEQ ID NO:32 (1551)	TAGCCTGGTCTAGCATGCTACCTGTACAAGCAGAAGGCACAACAAA		
SEQ ID NO:34 (1551)	TAGCCTGGTCTAGCATGCTACCTGTACAAGCAGAAGGCACAACAAA		
SEQ ID NO:36 (1551)	AAGTCTGGGTTAACATGTTACCTGTACAAGCAGAAGGCACAACAAA		
SEQ ID NO:5 (1551)	CAGCCTGGTGTGGCTGCTACCTGTACAAGCAGAAGGCCAGCAGA		
	1601		1650
SEQ ID NO:1 (1601)	AAACCCCTGCTGTGGCTGGCAACAAACACCCCTGGACCAGATGAGAGGCCACC		
SEQ ID NO:3 (1601)	AGACCTTGCTATGGCTTGGGAATAATACCCCTCGATCAGATGAGAGGCCACT		
SEQ ID NO:32 (1601)	AGACCTTGTTATGGCTTGGGAATAATACCCCTGATCAGATGAGAGGCCACT		
SEQ ID NO:34 (1601)	AGACCTTGTTATGGCTTGGGAATAATACCCCTGATCAGATGAGAGGCCACT		
SEQ ID NO:36 (1601)	AGACCTTGCTATGGCTTGGGAATAATACCCCTCGATCAGATGAGAGGCCACT		
SEQ ID NO:5 (1601)	AAACCCCTGCTGTGGCTGGCAATAACACCCCTGGACCAGATGAGGGCCACC		
	1651	1665	
SEQ ID NO:1 (1651)	ACCAGAGCCTGATGA		
SEQ ID NO:3 (1651)	ACAAGAGCATGA---		
SEQ ID NO:32 (1651)	ACAAAAATATGA---		
SEQ ID NO:34 (1651)	ACAAAAATATGA---		
SEQ ID NO:36 (1651)	ACAAGAGCATGA---		
SEQ ID NO:5 (1651)	ACCAGAACCTGATGA		

	SEQ ID NO:1	SEQ ID NO:3	SEQ ID NO:32	SEQ ID NO:34	SEQ ID NO:36	SEQ ID NO:5
SEQ ID NO:1		72%	71%	71%	71%	92%
SEQ ID NO:3			88%	89%	98%	69%
SEQ ID NO:32				99%	88%	70%
SEQ ID NO:34					88%	71%
SEQ ID NO:36						69%
SEQ ID NO:5						

Figure 13 (continued)

Protein sequence alignment of IBDV VP2

	1	50
SEQ ID NO:8	(1) MTNLQDQTQQIVPFI	RSLLMPTTGPASIPDDTLEKHTL
SEQ ID NO:42	(1) MTNLQDQTQQIVPFI	RSLLMPTTGPASIPDDTLEKHTL
	51	100
SEQ ID NO:8	(51) DTGSGLIVFFPGFPGSIVGAHYTLQ	SNGNYKFDQMLTAQNL
SEQ ID NO:42	(51) DTGSGLIVFFPGFPGSIVGAHYTLQ	SNGNYKFDQMLTAQNL
	101	150
SEQ ID NO:8	(101) LVSRSLT	VRSSTLPGGVYALNGTINAVTFQ
SEQ ID NO:42	(101) LVSRSLT	VRSSTLPGGVYALNGTINAVTFQ
	151	200
SEQ ID NO:8	(151) INDKIGNVLVGE	GVTVLSLPTSYDLGYVRLGDP
SEQ ID NO:42	(151) INDKIGNVLVGE	GVTVLSLPTSYDLGYVRLGDP
	201	250
SEQ ID NO:8	(201) DRPRVYTITAADDYQFSSQYQP	GGVTITLEFSANIDAITSLSIG
SEQ ID NO:42	(201) DRPRVYTITAADNYQFSSQYQT	GGVTITLEFSANIDAITSLSVG
	251	300
SEQ ID NO:8	(251) SVQGLVLGATIYLIGFDGTAVI	TRAVAADNGLTAGTDNLMPFN
SEQ ID NO:42	(251) SVQSLVLGATIYLIGFDGTAVI	TRAVAANNGLTAGIDNLMPFN
	301	350
SEQ ID NO:8	(301) ITQPITSIKLEIVTSKSGGQAGD	QMSWSASGSLAVTIHGGNYPGAL
SEQ ID NO:42	(301) ITQPITSIKLEIVTSKSDGQAGE	QMSWSASGSLAVTIHGGNYPGAL
	351	400
SEQ ID NO:8	(351) LVAYERVATGSVVTVAGVSN	FELIPNPELAKNLVTEYGRFD
SEQ ID NO:42	(351) LVAYERVATGSVVTVAGVSN	FELIPNPELAKNLVTEYGRFD
	401	450
SEQ ID NO:8	(401) ILSERDRIGIKTVWP	TREYTFREYFMEVADLNSPLKIA
SEQ ID NO:42	(401) ILSERDRIGIKTVWP	TREYTFREYFMEVADLNSPLKIA
	451	
SEQ ID NO:8	(451) IRR-	
SEQ ID NO:42	(451) IRR-	

SEQ ID NO:8 is 98% identical to SEQ ID NO:42

Figure 13 (continued)

DNA sequence alignment of IBDV VP2 gene

		1		50
SEQ ID NO: 7	(1)	ATGACAAACCTGCAAGATCAAACCCAACAGATTGTTCCGTTCATACGGAG		
SEQ ID NO: 41	(1)	ATGACAAACCTGCAAGATCAAACCCAACAGATTGTTCCGTTCATACGGAG		
		51		100
SEQ ID NO: 7	(51)	CCTTCTGATGCCAACAAACCGGACC GGCGTCCATTCCGGACGACACCCCTGG		
SEQ ID NO: 41	(51)	CCTTCTGATGCCAACAAACCGGACC GGCGTCCATTCCGGACGACACCCCTGG		
		101		150
SEQ ID NO: 7	(101)	AGAAGCACACTCTCAGGTCAAGAGACCTGACCTACAATTGACTGTGGGG		
SEQ ID NO: 41	(101)	AGAAGCACACTCTCAGGTCAAGAGACCTGACCTACAATTGACTGTGGGG		
		151		200
SEQ ID NO: 7	(151)	GACACAGGGTCAGGGCTAATTGTCCTTTCCCTGGATTCCCTGGCTCAAT		
SEQ ID NO: 41	(151)	GACACAGGGTCAGGGCTAATTGTCCTTTCCCTGGATTCCCTGGCTCAAT		
		201		250
SEQ ID NO: 7	(201)	TGTGGGTGCTCACTACACACTGCAGAGCAATGGGAACCTACAAGTTCGATC		
SEQ ID NO: 41	(201)	TGTGGGTGCTCACTACACACTGCAGAGCAATGGGAACCTACAAGTTCGATC		
		251		300
SEQ ID NO: 7	(251)	AGATGCTCCTGACTGCCAGAACCTACCGGCCAGCTACAACACTGCAGA		
SEQ ID NO: 41	(251)	AGATGCTCCTGACTGCCAGAACCTACCGGCCAGCTACAACACTGCAGG		
		301		350
SEQ ID NO: 7	(301)	CTAGTGAGTCGGAGTCTCACAGTGAGGTCAAGCACACTCCCTGGTGGCGT		
SEQ ID NO: 41	(301)	CTAGTGAGTCGGAGTCTCACAGTGAGGTCAAGCACACTCCCTGGTGGCGT		
		351		400
SEQ ID NO: 7	(351)	TTATGCACTAACGGCACCATAAACGCCGTGACCTTCCAAGGAAGCCTGA		
SEQ ID NO: 41	(351)	TTATGCACTAACGGCACCATAAACGCCGTGACCTTCCAAGGAAGCCTGA		
		401		450
SEQ ID NO: 7	(401)	GTGAAC TGACAGATTTAGCTACAATGGGTGATGTCGAAACAGCCAAC		
SEQ ID NO: 41	(401)	GTGAAC TGACAGATTTAGCTACAACGGGTGATGTCGAAACAGCCAAC		
		451		500
SEQ ID NO: 7	(451)	ATCAACGGACAAAATTGGGAATGTCCTGGTAGGGGAAGGGGTCACTGTCCT		
SEQ ID NO: 41	(451)	ATCAACGGACAAAATTGGGAACGGTCTAGTAGGGGAAGGGTAACCGTCCT		
		501		550
SEQ ID NO: 7	(501)	CAGCCTACCCACATCATGATCTGGGTATGTGAGGCTTGGTGACCCCCA		
SEQ ID NO: 41	(501)	CAGCTTACCCACATCATGATCTGGGTATGTGAGGCTTGGTGACCCCCA		
		551		600
SEQ ID NO: 7	(551)	TTCCCGCTATAAGGGCTTGACCCAAAAATGGTAGCTACATGCGACAGCACT		
SEQ ID NO: 41	(551)	TACCCGCTATAAGGGCTTGACCCAAAAATGGTAGCAACATGTGACAGCACT		

Figure 13 (continued)

		601	650
SEQ ID NO:7	(601)	GACAGGCCAGAGTCTACACCATAACTGCAGCCGATGATTACCAATTCTC	
SEQ ID NO:41	(601)	GACAGGCCAGAGTCTACACCATAACTGCAGCCGATAATTACCAATTCTC	
		651	700
SEQ ID NO:7	(651)	ATCACAGTACCAACCAGGTGGGTAACAATCACACTGTTCTCAGCCAACA	
SEQ ID NO:41	(651)	ATCACAGTACCAAACAGGTGGGTAACAATCACACTGTTCTCAGCCAACA	
		701	750
SEQ ID NO:7	(701)	TTGATGCTATCACAAGCCTCAGCATTGGGGAGAGCTCGTGTTCAAACA	
SEQ ID NO:41	(701)	TTGATGCCATCACAAGTCTCAGCGTTGGGGAGAGCTCGTGTTCAAACA	
		751	800
SEQ ID NO:7	(751)	AGCGTCCAAGGCCTTGTACTGGCGCCACCATCTACCTTATAGGCTTTGA	
SEQ ID NO:41	(751)	AGCGTCCAAGCCTTGTACTGGCGCCACCATCTACCTTATAGGCTTTGA	
		801	850
SEQ ID NO:7	(801)	TGGGACTGCGGTAATCACCAGAGCTGTAGCCGCAGATAATGGGCTGACGG	
SEQ ID NO:41	(801)	TGGGACTGCGGTAATCACCAGAGCTGTGGCGCAAACAATGGGCTGACGG	
		851	900
SEQ ID NO:7	(851)	CCGGCACCGACAATCTTATGCCATTCAATCTTGTCAATTCAAACCAATGAG	
SEQ ID NO:41	(851)	CCGGCATCGACAATCTTATGCCATTCAATCTTGTCAATTCAAACCAATGAG	
		901	950
SEQ ID NO:7	(901)	ATAACCCAGCCAATCACATCCATCAAACCTGGAGATAGTGACCTCCAAAAG	
SEQ ID NO:41	(901)	ATAACCCAGCCAATCACATCCATCAAACCTGGAGATAGTGACCTCCAAAAG	
		951	1000
SEQ ID NO:7	(951)	TGGTGGTCAGGCAGGGGATCAGATGTATGGTCGGCAAGTGGGAGCCTAG	
SEQ ID NO:41	(951)	TGATGGTCAGGCAGGGAACAGATGTATGGTCGGCAAGTGGGAGCCTAG	
		1001	1050
SEQ ID NO:7	(1001)	CAGTGACGATCCATGGTGGCAACTATCCAGGGGCCCTCCGTCCCGTCACA	
SEQ ID NO:41	(1001)	CAGTGACGATCCATGGTGGCAACTATCCAGGAGGCCCTCCGTCCCGTCACA	
		1051	1100
SEQ ID NO:7	(1051)	CTAGTAGCCTACGAAAGAGTGGCAACAGGATCCGTGTTACGGTCGCTGG	
SEQ ID NO:41	(1051)	CTAGTGGCCTACGAAAGAGTGGCAACAGGATCTGTGTTACGGTCGCTGG	
		1101	1150
SEQ ID NO:7	(1101)	GGTGAGTAACCTCGAGCTGATTCCAAATCCTGAACTAGCAAAGAACCTGG	
SEQ ID NO:41	(1101)	GGTGAGCAACCTCGAGCTGATCCAAATCCTGAACTAGCAAAGAACCTGG	
		1151	1200
SEQ ID NO:7	(1151)	TTACAGAATACGGCCGATTTGACCCAGGAGCCATGAACACTACACAAAATTG	
SEQ ID NO:41	(1151)	TTACAGAATATGGCCGATTTGACCCAGGAGCCATGAACACTACACGAAATTG	
		1201	1250
SEQ ID NO:7	(1201)	ATACTGAGTGAGAGGGACCGTCTGGCATCAAGACCGTCTGGCCAACAAG	
SEQ ID NO:41	(1201)	ATACTGAGTGAGAGGGACCGCCTGGCATCAAGACCGTCTGGCCAACAAG	

Figure 13 (continued)

	1251		1300
SEQ ID NO:7 (1251)	GGAGTACACTGATTTCGTGAGTACTTCATGGAGGTGGCCGACCTCAACT		
SEQ ID NO:41 (1251)	GGAGTACACTGACTTCGTGAGTACTTCATGGAGGTGGCCGACCTCAACT		
	1301		1350
SEQ ID NO:7 (1301)	CTCCCCCTGAAGATTGCAGGAGCATTGGCTTCAAAGACATAATCCGGGCT		
SEQ ID NO:41 (1301)	CTCCCCCTGAAGATTGCAGGAGCATTGGCTTCAAAGACATAATCCGGGCC		
	1351	1362	
SEQ ID NO:7 (1351)	ATAAGGAGGTAA		
SEQ ID NO:41 (1351)	ATAAGGAGGTGA		

SEQ ID NO:7 is 97% identical to SEQ ID NO:41

Figure 14

DNA and protein sequences

NDV-F codon optimized DNA (SEQ ID NO:1)

atggcagcaagccagcacaagaatcccagccccctgatgtatcacccgcacatcat
gctgatcctggctgatcagacccacaagactccctggatggacgccccctggccgctg
ccggcatcgttgtgaccggcgacaaggccgtgaacgttacaccagcagccagaccggc
agcatcatcgtgaagctgctgccaacatgcccagagacaagaggcctgogccaaggc
ccccctgaaaggcatacacaacagaaccctgaccaccctgctgaccccccctggcgacagca
tcagaaaagatccagggtccgtgagcacaagcggcggaggaagcagggcagactgatc
ggccgtgtatcggcagcgtggccctggagtggctacagctgcccagattaccgctgc
agcccccgtatccaggccaaccagaacgcccacatcctgagactgaaagagagca
ttgcgcaccaacgaggccgtgacgaagtgaccgacggcctgagccagctgtccgtg
gccgtggcaagatgcagcagttcgtgaacgaccagtcaacaacaccgcccagagagct
ggactgcatcaagatcaccaggcaggtggcgatggactgaaacctgtacctgaccgac
tgaccacagtgtccggccccagatcacaagccagccctgacacagctgaccatccag
gccctgtacaacctggctggcgcaacatggactatctgctgacaaagctggaaatcg
caacaaccagctgtccagcctgatcggaaagcggcctgatcaccggctacccatcctgt
acgacagccagacacagctgtggcatccaggtaacctgcccagcgtggcaacctg
aacaacatgcgcgcacacctacctggaaaccctgagcgtgtccaccaccaaggctacgc
cagccctgtggcccaagggtggacacaggtggcagcgtgatcgaggaactggaca
ccagctactgcatcgagagcgcacctggacctgtactgcaccagaatcgtgaccttcca
atgagcccgcatctacagctgctgagcggcaacaccagcgcctgatgtacagcaa
gaccgaaggcgcactgacaacaccctacatggccctgaaaggaaagcgtgatcgccaact
gcaagatcaccacctgcagatgcaccgaccccccaggcatcatcagccagaactacggc
gaggccgtgagcctgatcgatcgcattcctgtaacgtgctccctggacggcatcac
actgagactgagcggcgagttcgatgccacctaccagaagaacatcagcatcctggaca
gccaggtgatcgtgaccggcaacctggacatcagcaccgagctggcaacgtgaataac
agcatcagcaacgcctggacagactggccgagagcaacagcaagctggaaaaagtgaa
cgtgcgcctgacatccacttccgctctgatcacctacatcgtgctgaccgtgatcagcc
tggtgtccggccctgagcctgtgctggctgctacctgatgtacaagcagaaggcc
cagcagaaaaccctgctgtggctggcaacaacaccctggaccagatgagagccaccac
cagagcctgatga

NDV-F protein (SEQ ID NO:2)

MGSKPSTRIPAPLMLITRIMLILGCIIRPTSSLDRPLAAAGIVVTGDKAVNVYTSSQTGSIIIVKL
LPNMPRDKEACAKAPLEAYNRTLTLTTPLGDSIRKIQGSVSTSGGGKQGRLIGAVIGSVALGVA
TAAQITAAAALIQQANQNAANILRLKESIAATNEAVHEVTGDSQLSVAVGKMQQFVNQDFNNTAR
ELDCIKITQQGVVELNLYLTELTTVFGPQITSALTQLTIQALYNLAGGNMDYLLTKLGIGNNQL
SSLIGSGLITGYPILYDSQTQLLGIVQNLPVGVLNNMRATYLETLSVSTTKGYASALVPKVVTQ
VGSVIEELDTSYCIESDLDLYCTRIVTFPMSPGIYSCLSGNTSACMYSKTEGALTPYMALKGSV
IANCKITTCCRCTDPPGIISQNYGEAVALRHSNCVLSLDGITLRLSGEFDATYQKNISIILDSQV
IVTGNDISTELGNVNNISNALDRLAESNSKLEKVNVRLTSTSALITYIVLTVISLVFGALSIV
LACYLMYKQKAQQKTLWLGNNTLDQMRATTR*

Figure 14 (Continued)

DNA sequence of NDV-F VII^d wildtype (SEQ ID NO:3)

atgggctccaaaccttctaccaggatcccagcacctctgatgctgatcacccggattat
 gctgatattggctgtatccgtccgacaagctctttgacggcaggccttgcagctg
 caggaattgttagtaacaggagataaggcagtcaatgtatacacttcgtctcagacaggg
 tcaatcatagtcaagtgctccgaatatgcccaggataaggaggcgtgtgaaaagc
 cccattagaggcatataacagaacactgactactttgctcactcctcttggcactcca
 tccgcaagatccaagggtctgtgtccacatctggaggaggcaagcaaggccctgata
 ggtctgttattggcagttagtcttgggttcaacagcggcacagataacagcage
 tgccgcctaatacaagccaaccagaatgccgccaacatcctccggcttaaggagagca
 ttgctgcaaccatgaagctgtcatgaagtcaccgacggattatcacaactatcagtg
 gcagttggaaagatgcagcagttgtcaatgaccagttataatacggcgagaaatt
 ggactgtataaaaatcacacaacacaggttggtagaactcaacctatacctaactgaat
 tgactacagtattcggccacagatcacccctgcattaactcagtcgaccatccag
 gcacttataatttagtggcaatatggattacttactaactaagtttaggtatagg
 gaacaatcaactcagctcgtaattggtagcggcctgatcactggttaccctatactgt
 atgactcacagactcaactcttggcatacaagtgaatttaccctcagtcggaaactta
 aataatatgcgtgccacctattggagaccttatctgtaagtacaaccaaaggatatgc
 ctcagcacttgcggaaagttagtgcacacaagtgcgttccgtatagaagagactgaca
 cctcatactgtatagagtccgatctggatttatattgtactagaatagtgcattcccc
 atgtccccaggattttattcctgtttgagcggcaacacatcagttgcatttgcattcaaa
 gactgaaggcgcactcactacgcccgtatatggcccttaaggctcagttattgccaatt
 gtaaaaataacaacatgttagatgtacagaccctcctggtatcatatgcaaaaattatgga
 gaagctgtatccctgatagatagacattcgtcaatgtcttattcattagacggataac
 tctaaggctcagtgccggaaatttgcatttgcacttataactcaataacttagatt
 ctcaagtcatcgtgcacaggcaatcttgcattatcaactgaacttggaaacgtcaacaat
 tcaatcagcaatgcctggataggttggcagaaagaacacagcaagctagaaaaagtcaa
 tgtcagactaaccagcacatctgcatttgcatttgcatttgcatttgcatttgcatttgc
 tagtttcggtgacttagtctggtagtgcgttgcatttgcatttgcatttgcatttgcatttgc
 caacaaaagaccttgcattggcttggataataccctcgatcagatgagagccactac
 aagagcatga

Amino Acid sequence of NDV-F VII^d wildtype (SEQ ID NO:4)

1	MGSKPSTRIP APLMLITRIM LILGCIRPTS SLDGRPLAAA GIVVTGDKAV
51	NVYTSSQTGS IIVKLLPNMP RDKEACAKAP LEAYNRTLTT LLTPLGDSIR
101	KIQGSVSTSG GGKQGRLIGA VIGSVALGVA TAAQITAAAA LIQANQNAAN
151	ILRLKESIAA TNEAVHEVTD GLSQLSVAVG KMQQFVNQDF NNTARELDCI
201	KITQQVGVEL NLYLTELTTV FGPQITSPAL TQLTIQALYN LAGGNMDYLL
251	TKLGIGNNQL SSLIGSGLIT GYPILYDSQT QLLGIQVNLP SVGNLNMMRA
301	TYLETLSVST TKGYASALVP KVVTQVGSVI EELDTSYCIE SDLDLYCTRI
351	VTFPMSPGIY SCLSGNTSAC MYSKTEGALT TPYMAKGCSV IANCKITTCR
401	CTDPPGIISQ NYGEAVSLID RHSCNVLSLD GITLRLSGEF DATYQKNISI
451	LDSQVIVTGN LDISTELGNV NNSISNALDR LAESNSKLEK VNVRLTSTSA
501	LITYIVLTVI SLVFGALSLV LACYLMYKQK AQQKTLWLWLG NNTLDQMRAT
551	TRA*

Figure 14 (Continued)

DNA sequence of NDV-F-CAO2-CSmut (SEQ ID NO:5) for HVT116

atgggcagcaagcccacgtcacctggatcagcgtgaccctgatgtatcaccagaaccat
 gctgatcctgagctgcatactgccccacaaggcgcacgcgcggcggacggccctggccgctg
 ccggcatcggtgaccggcgacaaggccgtgaacatctacaccaggcagccagaccggc
 agcatcatcatcaagctgtgccaacatgccaaggacaaagaggcctgcgcacaaggc
 cccctggaagcctacaacagaaccctgaccaccctgctgaccacccctggccgacagca
 tcagaagaatccaggcgccaccacaaggcggggaggaaagcaggcgagactggtg
 ggcgctatcatcgggagcgtggccctggcgtggccacagctgcccagattaccgctgc
 agccgcctgattcaggcaatcagaacgcgcacatcctgagactgaaagagagca
 ttggccaccaccaacgcacgcgtgcacgaatgacaaacggactgtccagctggctgtc
 gctgtcgcaagatgcagcagtctgtgaacaaccaggtaacaacaacaccgcagagagct
 ggactgcataagatgcgcgcaggcggctggcgtggagctgaaacctgtacctgaccgagc
 tgaccacagtgttcggcccccagatcacaagccccgtctgaccaggctgacaatccag
 gccctgtacaaccctggctggcgcaacatggactatctgctgactaagctggagttgg
 caacaaccaggctgtccagcctgatcgggtccggcgtgatcacaggcaacccatcctgt
 acgacagccagacacagctgtggcatccagatcaaccctgcccattggagctgaaacctg
 aacaacatgagagccacccatcggaaaccctgagcgtgtccaccaccaaggcgttcgc
 cagcgcctggcggccaaagggtggtacacaggcggcagcgtgatcgaggaactggaca
 ccagctactgcatacgagcgcacatcgacctgtactgcaccaggctggatggcatccca
 atgagccccggcatctacagctgcctgagcggcaacaccaggcgcctgatgtacagcaa
 gaccgaaggaggactgacaacaccctacatggccctgaaggaaagcgtgatgcacaact
 gcaagatgaccacactgcagatgcgcgcaccctggcatcatcagccagaactacggc
 gagggcgtgagcctgatcgacaaacattcctgttagcgtgctgtccctggatggcatcac
 actgagactgagcggcggacttcgacgcacccatcggcgtgatgtacagcatcctggaca
 gccagggtatcgtgacggcaacctggacatcagcaccaggctggcaacgtgaacaac
 agcatcagcagcaccctggacaagctggcggacttccaaacaaggctgatcggaaatgaa
 cgtgaacctgaccaggcacaaggcgcctgatcacctacatcgtgctggccatcgtgtccc
 tggccttcggcgtgatcgcctggctgctggcctgctacatcgtgatgtacaaggcagagagc
 cagcagaaaaaccctgctgtggctggcaataacaccctggaccaggatgagggccaccac
 cagaacctgtatga

Amino Acid sequence of NDV-F-CAO2-CSmut (SEQ ID NO:6) for HVT116

1	MGSKPSTWIS VTLMLITRTM LILSCICPTS SLDGRPLAAA GIVVTGDKAV
51	NIYTSSQTGS IIIKLLPNMP KDKEACAKAP LEAYNRTLTT LLTPLGDSIR
101	RIQGSATTSG GGKQGRLVGA IIIGSVALGVA TAAQITAAAA LIQANQNAAN
151	ILRLKESIAA TNDAVHEVTN GLSQLAVAVG KMQQFVNQNF NNTARELDCI
201	KIAQQVGVEL NLYLTELTTV FGPQITSPAL TQLTIQALYN LAGGNMDYLL
251	TKLGVGNNQL SSLIGSGLIT GNPILYDSQT QLLGIQINLP SVGSLNNNMRA
301	TYLETLSVST TKGFASALVP KVVTQVGSVI EELDTSYCIE SDIDLYCTRV
351	VTFPMSPGIY SCLSGNTSAC MYSKTEGALT TPYMALKGSV IANCKMTTCSR
401	CADPPGIISQ NYGEAVSLID KHSCSVLSLD GITLRLSGEF DATYQKNISI
451	LDSQVIVTGN LDISTELGNV NNSISSTLDK LAESNNKLNK VNVLNTSTSA
501	LITYIVLAIIV SLAFGVISLV LACYLMYKQR AQQKTLWLIG NNTLDQMRAT
551	TRT*

Figure 14 (Continued)

DNA coding for IBDV VP2 protein (SEQ ID NO:7)

ATGACAAACCTGCAAGATCAAACCCAACAGATTGTTCCGTTCATACGGAGCCTCTGAT
GCCAACAAACCGGACCGCGTCCATTCCGGACGACACCCTGGAGAAGCACACTCTCAGGT
CAGAGACCTCGACCTACAATTGACTGTGGGGACACAGGGTCAGGGCTAATTGTCTTT
TTCCCTGGATCCCTGGCTCAATTGTGGGTGCTCACTACACACTGCAGAGCAATGGGAA
CTACAAGTTCGATCAGATGCTCCTGACTGCCAGAACCTACCGGCCAGCTACAACACT
GCAGACTAGTGAGTCGGAGTCTCACAGTGAGGTCAAGCACACTCCCTGGTGGCGTTAT
GCACTAAACGGCACCATAAACGCCGTGACCTCCAAGGAAGCCTGAGTGAACGTGACAGA
TGTTAGCTACAAATGGGTGATGTCTGCAACAGCCAACATCAACGACAAAATTGGAATG
TCCTGGTAGGGGAAGGGGTCACTGTCCTCAGCCTACCCACATCATATGATCTGGGTAT
GTGAGGCTTGGTGACCCCATTCCCGCTATAGGCTTGACCCAAAATGGTAGCTACATG
CGACAGCAGTGACAGGCCAGAGTCTACACCATAACTGCAGCCGATGATTACCAATTCT
CATCACAGTACCAACCAGGTGGGTAACAATCACACTGTTCTCAGCCAACATTGATGCT
ATCACAAGCCTCAGCATTGGGGAGAGCTCGTGTTCAAACAAGCGTCCAAGGCCTTGT
ACTGGCGCCACCATCTACCTTATAGGCTTGATGGACTGCGGTAATCACCAGAGCTG
TAGCCGAGATAATGGCTGACGGCCGGCACCGACAATCTTATGCCATTCAATCTGTC
ATTCCAACCAATGAGATAACCCAGCCAATCACATCCATCAAACCTGGAGATAGTGACCTC
CAAAGTGGTGGTCAGGCAGGGATCAGATGTCATGGTGGCAAGTGGGAGCCTAGCAG
TGACGATCCATGGTGGCAACTATCCAGGGCCCTCCGTCCCGTACACTAGTAGCCTAC
GAAAGAGTGGCAACAGGATCCGTCGTTACGGTCGCTGGGTGAGTAACTCGAGCTGAT
TCCAAATCTGAACTAGCAAAGAACCTGGTTACAGAATACGGCGATTGACCCAGGAG
CCATGAACTACACAAAATTGATACTGAGTGAGAGGGACCGTCTGGCATCAAGACCGTC
TGGCCAACAAGGGAGTACACTGATTTCGTGAGTACTTCATGGAGGTGGCGACCTCAA
CTCTCCCCTGAAGATTGCAGGAGCATTGGCTCAAAGACATAATCCGGCTATAAGGA
GGTAA

IBDV VP2 protein (SEQ ID NO:8)

MTNLQDQTQQIVPFIRSLLMPPTGPASIPDDTLEKTLRSETSTYNLTVGDTGSGLIVF
FPFGFPGSIVGAHYTLQSNGNYKFDQMLLTAQNLPASYNYCRLVRSLSLTVRSSTLPGGVY
ALNGBTINAVTFQGSLSLETDVSYNGLMSATANINDKIGNVLVGEVTVLSPSYDLGY
VRLGDPPIAIGLDPKMVATCDSSDRPRVYTITAADDYQFSSQYQPAGVTITLFSANIDA
ITSLSIGGELVFQTSVQGLVLGATIYLIGFDGTAIVTRAVALDNGLTAGTDNLMPFNLV
IPTNEITQPITSIKLEIVTSKSGGQAGDQMSWSASGSLAVTIHGNYPGALRPVTLVAY
ERVATGSVVTVAGVSNFELIPNPELAKNLTVEYGRDPGAMNYTKLILSERDRLGIKTV
WPTREYTDFFREYFMEVADLNSPLKIAGAFGFKDIIRAIRR

Sv40 Promoter (SEQ ID NO:9)

gaattcgagctcggtacagcttggctgtggaatgtgtgtcagttagggtgtggaaagtcc
cccaggctccccagcaggcagaagtatgcaagcatgcatttcatttcgcacca
ggtgtggaaagtccccaggctccccagcaggcagaagtatgcaagcatgcatttc
tagtcagcaaccatagccccccctaactccgcctatccgccttaactccgcctc
ttccgccttccgccttgcactaatttttatgtcagaggccgagg
ccgcctcggcctctgagctattccagaagtatgtcaggaggcttttgaggccatggc
tttgcaaaaagct

Figure 14 (Continued)

CMV-IE promoter (SEQ ID NO:10)

aactccggccgtttatgactagaaccaatagtttaatgccaaatgcactgaaatcc
cctaatttgc当地aagccaaacgc当地ccatgtgactgtacatcgaaaactttaccataatt
tcccaaggccaaagccccc当地ataactcatatggcatatqaatcagcacggctatgca
ctctaattggccgccc当地aggacttccacataggggcgltcaccattccc当地cata
ggggggactcaatggc当地taccatggactatgggtcaatggaggtaaagccaaat
gggtttcc当地acttggcaagcacactgactgactcaaatggactttccactggg當地t
ccaaagtacattgggtcaatggaggtgagccaatggaaaaccattgtc当地caag
acactgactacaataggactttcaatgggactttccattgttctgccc当地tacaagg
caatgtgggtgactcaataggactttccattgttctgccc当地tacaagg
aggggtaatcaacaggaaagtccc当地tggagccaactacactgc当地tcaatagg
ttccattggg当地tggcc当地tactaaggcaataggggatgactgactcaatgg
cattggagccaacttgc当地tactcaataggactttccattggg当地tggcc当地t
aaggcaataggggacttgc当地tactcaacaggaaagtccc当地tggagccaactt
ataggactttccatggg当地tggcc当地tactgacttcaataggactttccatgg
gggtt当地tcc当地tactggacttgc当地tactcaataggactttccatgg
ccactaaggcaatagggtgaatcaacaggaaagtccc当地tggagccaactt
ataggactttccatggg当地tggcc当地tactgacttcaataggactttccatgg
gggtt当地tcc当地tactggacttgc当地tactcaataggactttccatgg
actcaatggg当地tggcc当地tacttggacttgc当地tactcaatagg
gggtt当地tcc local attaattaaaacggccatgtacttccaccattgacgtcaatgg
gctattgaaactaatgcaacgtgaccttaacggacttccatagctgattaatgg
gaaagtaccgttctcgagccaatcacgtcaatgggaaagtgaaagggcagccaaacgt
aacaccggcccggttccc当地tggaaattccatattggcacgcattctattgg
tgc当地tctactggtataagaggc当地tgc当地tactgctgacttgc当地t
tgaccaccgtagaacgc当地tctcgact

SV40 polyA signal (SEQ ID NO:11)

Ggggatccagacatgataagatatacttgc当地tggacaaccacaactagaatgca
gtgaaaaaaaatgctt当地tgc当地tggaaattttgtgactgtacttgc当地tatttgc当地t
taagctgcaataaacaacttaacaacaacaattgc当地tggacttgc当地tattttatgc当地t
ggggagggtgtgggagggtt当地tggatcc当地tactgact

Synthetic polyA signal (SEQ ID NO:12)

aataaaaatctt当地tatttgc当地tattacatctgtgtggtggg当地tgg
actaacatc当地tgc当地tctc当地tcatcaaaacaacaacaacttagcaaaaatagg
tccc当地tgc当地tactgactgactgc当地tggccacaacattctctt

Figure 14 (Continued)

The nucleotide sequence of the cloned NDV Texas F gene (wild type non-modified) (SEQ ID NO:32)

ATGGGCTCCAGATCTTCTACCAGGATCCCGGTACCTCTAATGCTGATCATCCGAACCGC
GCTGACACTGAGCTGTATCCGCTGACAAGCTCTCTTGATGGCAGGCCCTTGCAGCTG
CAGGGATCGTGGTAACAGGAGATAAACAGTCACATACACCTCATCCCAGACAGGG
TCAATCATAGTTAAGTTACTCCGAATATGCCAAGGACAAAGAGGTGTGCAAAGC
CCCATTGGAGGCATACAAACAGGACACTGACTACTTTACTCACCCCCCTGGTGATTCTA
TCCGCAGGATACAAGAGTCTGTGACTACTTCCGGAGGAAGGAGACAGAGACGCTTTATA
GGTGCCTTATCGGCAGTGTAGCTCTGGGTTGGACAGCTGCACAGATAACAGCAGC
TTCGGCCCTGATACAAGCCAACCAGAATGCTGCCAACATCCTCCGGCTAAAGAGAGCA
TTGCTGCAACCAATGAAGCTGTGACGAGGTCACTGACGGATTATCACAACTAGCAGTG
GCAGTAGGAAAGATGCAACAGTTGTCAATGACCAGTTCAATAATACAGCGCAAGAATT
GGACTGTATAAAAATTGCACAGCAGGTCGGTGTAGAACTCAACTTGTACCTAAGTGAAT
TGACTACAGTATTGGGCCACAAATCACTCCCTGCCTTAACTCAGCTGACTATCCAA
GCGCTTTACAATCTAGCTGGTGGTAATATGGATTACTGCTGACTAAGTTAGGTGTAGG
GAACAACCAACTCAGCTCATTAATTGGTAGCGGCTGATCACCGGCAACCTATTCTGT
ACGACTCACAGACTCAGATCTGGGTATACAGGTAACCTTGCCCTCAGTTGGAACCTG
AATAATATGCGTGCCACCTACCTGGAGACCTTATCTGTAAGCACAACCAAGGGATTG
CTCAGCACTTGTCCCCAAAGTGGTACACAGGTCGGTCCGTGATAGAAGAACTTGACA
CCTCATACTGTATAGGGACCGACTGGATTATACTGTACAAGAATAGTGCACATTCCCT
ATGTCTCCTGGTATTTATTCTGTCTGAGCGGTAAACATCGGCTTGATGTATTCAA
GACTGAAGGGCGACTTACTACGCCATATATGGCTCTAAAGGCTCAGTTATTGCCAATT
GCAAGCTGACAACATGTAGATGTGCAAGATCCCCCAGGTATCATATCGAAAATTATGGA
GAAGCTGTGTCCTTAATAGATAGGCACTCATGCAACGTCTTACCTAGACGGGATAAC
TCTGAGGCTCAGTGGGAATTGATGCAACCTATCAAAGAATATCTTACACTAGATT
CTCAAGTTATAGTGCACAGGCAATCTTGATATCAACTGAGCTTGGGAATGTCAACAAAC
TCAATAAGTAATGCCCTGAATAAGTTAGAGGAAAGCAACAGCAAACAGACAAAGTCAA
TGTCAAACGTGACCAGCACATCTGCTCTCATTACCTACATGTTAACTGTCATATCTC
TTGTTTTGGTACTTAGCCTGGTCTAGCATGCTACCTGATGTACAAGCAAAAGGCA
CAACAAAAGACCTTGTATGGCTGGGATAATACCCCTGATCAGATGAGAGCCACTAC
AAAAATATGA

The amino acid sequence of the cloned NDV Texas F gene (wild type non-modified; cleavage site underlined) (SEQ ID NO:33)

MGSRSSTRIPVPLMLIIRTALTLSCIRLTSSLDGRPLAAAGIVVTGDKAVNIYTSSQTG
SIIVKLLPNMPKDKEVCACKAPLEAYNRTLTLPLGDSIRRIQESVTTSGRRQRRFI
GAIIGSVALGVATAAQITAASALIQANQNAANILRLKESIAATNEAVHEVDGLSQLAV
AVGKMQQFVNNDQFNNTAQELDCIKIAQQVGVELNLYLTELTVFGPQITSPALTQLTIQ
ALYNLAGGNMDYLLTKLVGVNNQLSSLIGSGLITGNPILYDSQTQILGIQVTLPSVGNL
NNMRATYLETLSVSTTKGFASALVPKVVTQVGSVIEELDTSYCIGTDLDLYCTRIVTFP
MSPGIYSCLSGNTSACMYSKTEGALTPYMAKGSVIANCKLTTCRCADPPGIISQNYG
EAVSLIDRHSNCVLSLDGITLRLSGEFDATYQKNISILDSQVIVTGNLDISTEILGNVNN
SISNALNKLEESNSKLDKVNVLKTSTSALITYIVLTVISLVFGVLSVLACYLMYKQKA
QQKTLLWLGNNLTDQMRATTKI

Figure 14 (Continued)

NDV-F YZCQ wildtype DNA sequence (SEQ ID NO:34)

atgggctccagattttaccaggatcccgtaccttaatgctgatcatccgaaccgc
gctgacactgagctgtatccgtctgacaagctcttgcaggccttgcggctg
caggatcggttaacaggagataaaggcaactaacatatacacctatcccagacaggg
tcaatcatagttaaagttaactccgaatatgcccaaggacaaagaggtgtgcaaaagc
cccattggaggcataacaacaggacactgactactttactcaccccttggtgattcta
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ggtgcattatcgccagtgttagctcttgggttgcacagctgcacagataacacgc
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gaacaaccaactcagctcattaattggtagcggctgatcaccggcaaccctattctgt
acgactcacagactcagatcttgggtatacaggtaactttgccttcagttggAACCTG
aataatatgcgtgccacacctggagaccttatctgttaagcacaaccaagggttgc
ctcagcacttgccttccaaaatgtggacacaggtcggctgtatagaagaacttgaca
cctcataactgtataggaccgacttggattatactgtacaagaatagtgacattccct
atgtctcctggatttattctgtctgagcggtaatacatcggcttgcatttgcattcaaa
gactgaaggcgcacttactacgcctatatggctctcaaaggctcagtttgcatttgc
gcaagctgacaacatgttagatgtgcagatccccaggatcatatgcggaaaattatgg
gaagctgtgtccttaatagatagcactcatgcaacgtcttgcatttgcatttgcatttgc
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tgtcaaactgaccagcacatctgtctcattacctacatcggttactgtcatatctc
ttgttttgggttacttgcctgggttgcatttgcatttgcatttgcatttgcatttgc
caacaaaagaccttggcttgcatttgcatttgcatttgcatttgcatttgcatttgc
aaaaatatga

NDV-F protein from wildtype YZCQ strain (Amino Acid Sequence of NDV-F of Texas strain with lentogenic cleavage site sequence) (SEQ ID NO:35)

mgsrssstripvplmliirtaltlscirltssldgrplaaagivvtgdakavniytssqtg
siivkllpnmpkdkevcakapleaynrtlttltpulgdsirriquesvttsgggkqgrli
gaiigsvalgvataaqitaasaliqanqnaanilrlkesiaatneavhevtdglsqlav
avgkmqqfvndqfnntaqelcdciakiaqqvgvelnlyltelttvfgpqitspaltqltiq
alynlaggnmdylltklgvgnnqlsslsligsglitgnpilydsqtqilgiqvtlpsvgnl
nnmratylettsvsttkgfasalvpkvvtqvgsvieeldtsycigtdldlyctrivtfp
mspgiysclsqntsacmysktegaltpymalkgsviancklttcrcadppgiisqnyg
eavslidrhscnvlsldgitlrlsgefdatyqknisildsqvivtgnldistelgnvnn
sisnalnklesnskldkvnvkltstsalityivlvislvfgvslvlacylmykqka
qkctlwlgnntldqmrrattki*

Figure 14 (Continued)

NDV-F Texas wildtype DNA sequence (SEQ ID NO:36)

atgggctctaaaccccttaccaggatcccagcacccctgtatgcgtatcaccggattat
gctgatattggactgtatccgtccgacaagctctttgacggcaggcctttgcagctg
caggaatttagtaacaggagataaggcagtcaatgtatatacctcgctcagacaggg
tcaatcatagtcaagtgtctccgaatatgcccaaggataaggaggcgtgtgcgaaaga
cccattagaggcatataacagaacactgactactttgctactcctcttggcgaatcca
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gcagttggaaagatgcagcagttgtcaatgaccagttataatacagcgcgagaatt
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gcactttataatattgtggcaatatggattacttattaactaagtttaggtatagg
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caacaaaagaccttgcataggcttggaaataataccctcgatcagatgagagccactac
aagagcatga

NDV-F protein from wildtype Texas strain (Amino Acid Sequence of NDV-F VII^d wt YZCQ with lentogenic cleavage site sequence) (SEQ ID NO:37)

mgskpstripaplmltrimlildcirptssldgrplaaagivvtgdakavnvytssqtg
siivkllpnmpkdkeacakdpleaynrtlttltpgesirkiqgsrstsgggkqgrli
gavigsvalgvataaqitaaaaliqanqnaanilrlkesiaatneavhevtdglsqlsv
avgkmqqfvndqfnntareldcikitqqvgvelnlyltelttvfgpqitspaltqltiq
alynlaggnmdyltklgignnqlssligrslitgypilydsqtqllgiqvnlpsvgnl
nnmratyletsvstakgyasalvpkvvtqvgsvieeldtsyciesdldlyctrivtfp
mspgiyisclsqntsacmysktegaltpymalkgsvianckittcrctdppgiisqnyg
eavslidrhscnvlsldgitlrlsgefdatyqknisisildsqvivtgndistelgnvnn
sisnaldklaksnsklekvnrsltstsalityivlvislvfgalslgltcylmykqka
qqktllwlgnntldqmrattra*

Figure 14 (Continued)

MDV gB promoter (SEQ ID NO:38)

CGATGTTAGTCACGATAGACATCGGTTGCCAGCCGTCGAATAACAGCATTATTTT
AGTGGTAAATGTAGGGCTGCTTCCTCACTTAAAGGAGGAATGGCTCGATTCATGTT
TCATAGCAGTAGAAAACAGATTGGACCGTCAGTAAGTTAGAGGGTTTATGACTTTA
GCACTATAGATAATGTAAC TGCGGCCATGCATGGCTGGAAATATCAAAGAACTG
ATTTTGCAACAGCTTATTTCTCTGTATTAAATGTGGCGAATTGCACATCTGTCG
TGCGCACAGTTGCAGATCAACAGCAATGGAGACTATGTATGGAAAATGGAATATATA
TAACATATGAAACCGAATATCCACTTATAATGATTCTGGGGTCAGAATCAAGCACTTCA
GAAACGCAAATATGACTGCAATTATTGATAACAGATGTTTCGTTGCTTATTCTAT
TTTGCAGTATATGGCCCCCGTTACGGCAGATCAGGTGCGAGTAGAACAGATTACCAACA
GCCACGCCCCCATCTGACCCGTCCAATATTCTGTGTCCTGCATTTATCTCACACAA
TTTATGAACAGCATCATTAAGATCATCTCACT

IBDV DNA encoding VP2 protein of IBDV E strain (SEQ ID NO:41)

atgacaaaacctgcaagatcaaaccacaaacagattgtccgttcatacgaggcctctgat
gc当地acaaccggaccggcgtccattccggacgacaccctggagaaggcacactctcaggt
cagagacctcgacctaatttactgtggggacacagggtcagggtcaattgtcttt
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tggccaaacaaggaggactgactgtttcggtgacttgcattggcggacccatcaa
ctctccctgaagattgcaggagcattggcttcaagacataatccggccataagga
ggta

Figure 14 (Continued)

IBDV VP2 protein of IBDV E strain (SEQ ID NO:42)

mtnlqdqtqqivpfirsllmpttgpasipddtlekhtlrsetstynltvgdtgsglivf
fpgfpqsvgahtlqsnngnykfdqmltaqnlpasynycrlvsrsltvrsstlpqgv
alngtinavtfqgslseltdvsynglmsatanindkignvlvgegtvlslptsydlgy
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iptneitqpitsikleivtsksdqqageqmswsasgslavtihgnypgalrpvtlvay
ervatgsvvttvagvsnfelipnpelaknlvteygrfdpgamnytklilsrdrlgiktv
wptreytdfreyfmevadlnsplkiagafgfkdriairr*

Guinea pig CMV promoter (SEQ ID NO:43)

ttagtcatatgttacttggcagaggcccatggaaagtccctggacgtggacatctga
ttaatacgtgaggaggtcagccatgttctttggcaaaggactacggtcattggacgt
ttgattggcatggatagggtcagccagagttAACAGTgttctttggcaaaggatac
gtggaaagtcccggccatttacagtaactgatacgggacaAAAGCACAGCCATT
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aatatgaaagaaggtcagccagaggttagctgtcctttggcaaaggatacggtt
tgggacgtttagtgactggatagggtcagccagagttAACAGTgttctttggcaa
aggaaaacgtgaaagtcccggccatttacagtaactgatactgggacaAAAGTACACC
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aggtgattcactgacatttggccgtcctctggaaagtccctggaaaccgctcaagtact
gtatcatggtgactttgcattttggagagcacgcggccactccaccatggtccacgta
ccctatggggagtggttatgagtatataaggggctccggtttagaagccggcaga

Figure 14 (Continued)

Partial plasmid pHM103+Fopt DNA sequence (SEQ ID NO:18)

Green and Italic = Arms

Black and bold = NDV Fopt

BLUE AND UPPERCASE = SV40 PROMOTER

Red and Italic and underlined = SV40 polyA

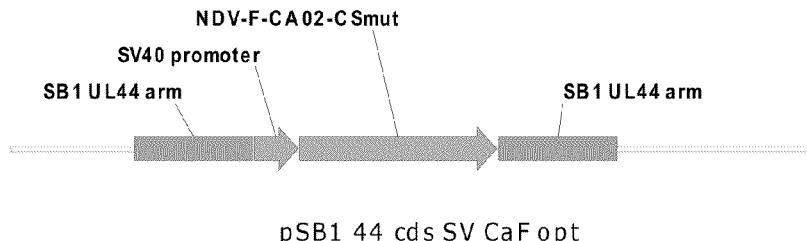
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AATT^tCGAGCTCGT^tTACAGCTGGCTGT^tGGAAATGTGT^tGTCAGTTAGGGTGT^tGGAA
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CAGCAACCAGGTGT^tGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAA
GCATGCATCTCAATTAGTCAGCAACCATA^tAGTCCC^tCCCTA^tACTCCGCC^tCATCC
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cagc^tag^tcc^tag^tcc^tgg^tgc^tag^tcat^tctgg^tgaa^tgt^tgc^tgt^tcc^tac^tg^tac^taga
caa^tag^tggc^tctgc^tcc^taa^tggccccctgg^tg^taa^tgc^tctaca^tac^taga^tac^tcc^tg^tacc^tac
cct^tgc^tg^tccccc^tctgg^tgc^tac^tg^tcat^tca^tgaaa^tat^tcc^tagg^tgc^tcc^tgt^tg^tag^tcc
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Figure 14 (Continued)

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gtaagaacaagccaaatgcacttccatattaacaagaagtgttagagagaataactc
aacctcttggatgtatcctcgag

Figure 14 (Continued)

**Partial plasmid pSB1 44cds SV FCAopt sequence for vSB1-009
(SEQ ID NO:19)**



Green and Italic = UL44 Recombination Arms
BLUE AND UPPERCASE = SV40 PROMOTER
Black and Bold = NDV-F-CA02-CSmut sequence

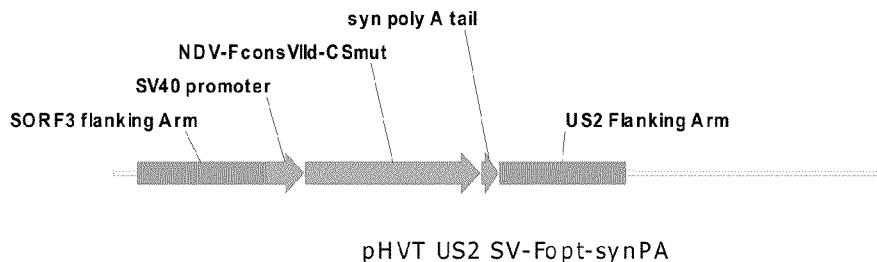
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Figure 14 (Continued)

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Figure 14 (Continued)

**Partial plasmid pHVT US2 SV-Fopt-SynPA for vHVT306
(SEQ ID NO:20)**



Green and Italic = SORF3 and US2 Recombination Arms

BLUE AND UPPERCASE = SV40 PROMOTER

Black and Bold = NDV-FconsVIId-CSmut sequence

Red and Italic and Underlined = Synthetic Poly A tail

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Figure 14 (Continued)

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Figure 14 (Continued)

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Figure 14 (Continued)

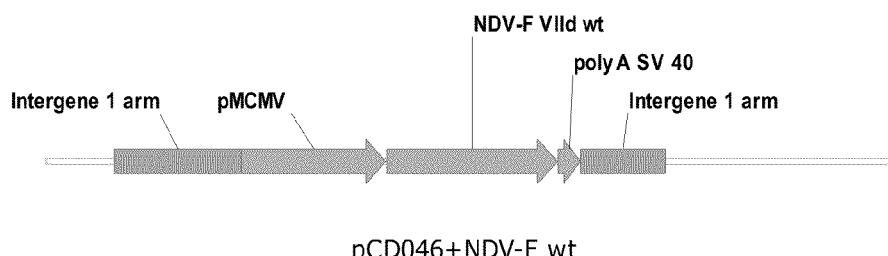
plasmid pCD046+NDV-F wt for vHVT110 (SEQ ID NO:21)

Green and Italic = BamHI fragment I intergenic Recombination Arms

BLUE AND UPPERCASE = MCMV PROMOTER

Black and Bold = NDV-F VII^d wildtype consensus sequence

Red and Italic and Underlined = SV40 Poly A tail



```

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Figure 14 (Continued)

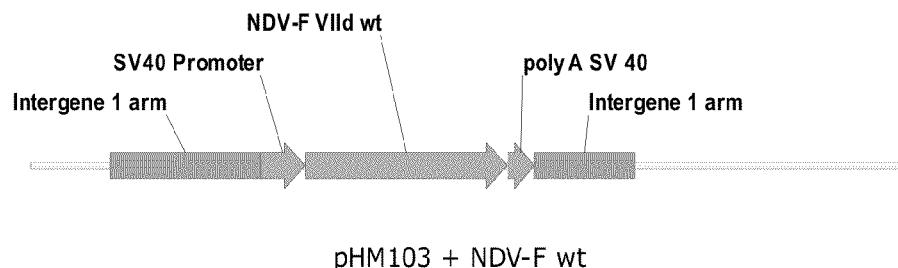
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Figure 14 (Continued)

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Figure 14 (Continued)

Partial plasmid pHM103+NDV-F wt sequence for vHVT111 (SEQ ID NO:22)



Green and Italic = BamHI fragment I intergenic Recombination Arms

BLUE AND UPPERCASE = SV40 PROMOTER

Black and Bold = NDV-F VIIId wildtype consensus sequence

Red and Italic and Underlined = SV40 Poly A tail

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Figure 14 (Continued)

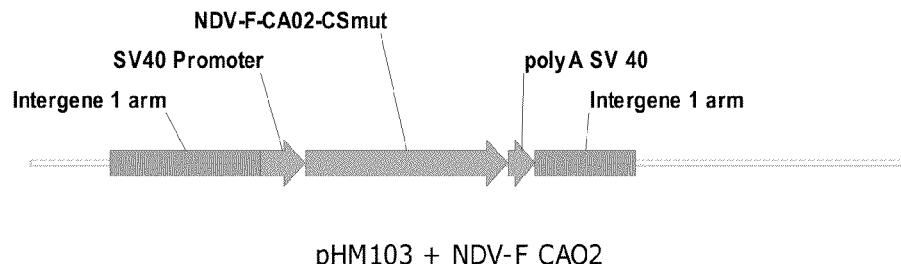
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Figure 14 (Continued)

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Figure 14 (Continued)

Partial plasmid pHM103+NDV-F CA02 for vHVT116 (SEQ ID NO:23)



Green and Italic = BamHI fragment I intergenic Recombination Arms

BLUE AND UPPERCASE = SV40 PROMOTER

Black and Bold = NDV-F-CA02-CSmut sequence

Red and Italic and Underlined = SV40 Poly A tail

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Figure 14 (Continued)

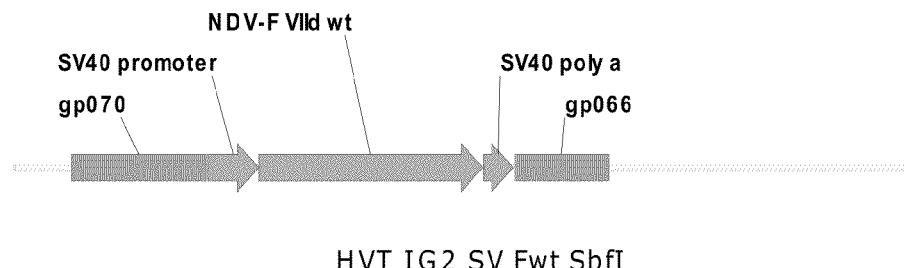
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Figure 14 (Continued)

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Figure 14 (Continued)

Partial plasmid HVTIG2 SV Fwt SbfI sequence for vHVT301 (SEQ ID NO:24)



*Green and Italic = gp070 and gp066 Recombination Arms
BLUE AND UPPERCASE = SV40 PROMOTER*

Black and Bold = NDV-F VIId wildtype consensus sequence
Red and Underlined = SV40 Poly A tail

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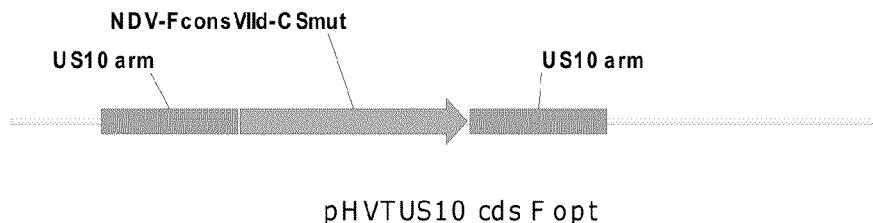
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Figure 14 (Continued)

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Figure 14 (Continued)

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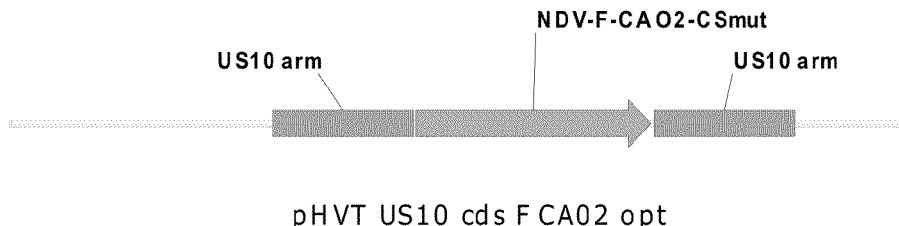


*Green and Italic = US10 cds Recombination Arms
Black and Bold = NDV-FconsVIId-CSmut sequence*

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Figure 14 (Continued)

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Figure 14 (Continued)**Partial plasmid pHVT US10 cds F CA02 opt sequence
for vHVT303 (SEQ ID NO:26)**

Green and Italic = US10 cds Recombination Arms
Black and Bold = NDV-F-CA02-CSmut sequence

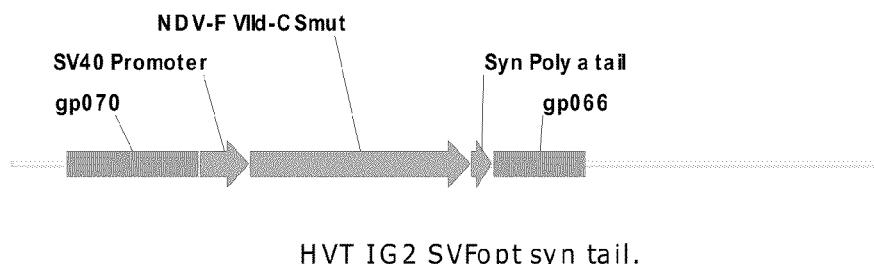
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Figure 14 (Continued)

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Figure 14 (Continued)

**Partial plasmid HVT IG2 SVFopt syn tail sequence
for vHVT304 (SEQ ID NO:27)**



Green and italic = gp070 and gp066 Recombination Arms

BLUE AND UPPERCASE = SV40 PROMOTER

Black and Bold = NDV-FconsVIId-CSmut sequence

Red and Underlined = Synthetic Poly A tail

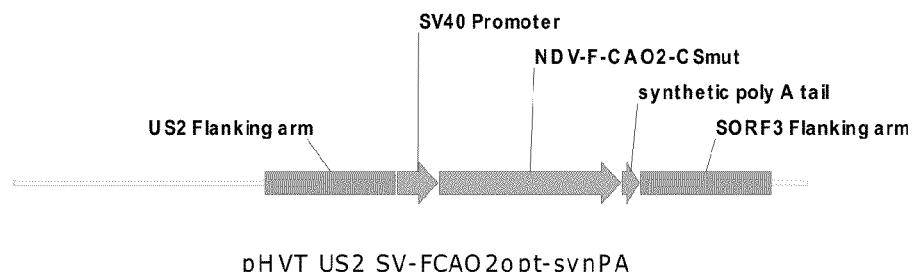
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Figure 14 (Continued)

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Figure 14 (Continued)

Partial plasmid pHVT US2 SV-FCA02 opt-synPA for vHVT307 (SEQ ID NO:28)



Green and Italic = US2 and SORF3 Recombination Arms

BLUE AND UPPERCASE = SV40 PROMOTER

Black and Bold = NDV-F-CAO2-CSmut sequence

Red and underlined = Synthetic Poly A tail

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Figure 14 (Continued)

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Figure 14 (Continued)

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Figure 14 (Continued)

Partial plasmid pCD046+NDV-F VII YZCQ for vHVT112 (SEQ ID NO:29)*Green and Italic = Flanking Arms**BLUE AND UPPERCASE = mCMV IE***Black and Bold = NDV-F VIIId wt YZCQ**Red and underlined = SV40 Poly A

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acGAATTCAATAGTGGATCCCCACTCCGCCGTTATGACTAGAACCAATAGTTT
TAATGCCAATGCACGTAACTCCCTAATTGCAAAGCCAAACGCCCTATGTGAGTA
ATACGGGGACTTTACCAATTCCCACGCCGAAAGCCCCCTAATACACTCATATGGC
ATATGAATCAGCACGGTCATGCACCTCAATGGCGGCCATAGGGACTTCCACATAGGG
GGCGTTCACCATTCCCAGCATAGGGTGGTGACTIONCAATGGCCTTACCCAAGTACATT
GGGTCAATGGGAGGTAAGCCAATGGGTTTCCCATTACTGGCAAGCACACTGAGTCAA
ATGGGACTTTCACTGGGTTTGCCTAAGTACATTGGGTCAATGGGAGGTGAGCCAATG
GGAAAAACCCATTGCTGCCAAGTACACTGACTCAATAGGGACTTCCAATGGGTTTTC
CATTGTTGGCAAGCATATAAGGTCAATGTGGGTGAGTCAATAGGGACTTCCATTGTAT
TCTGCCAGTACATAAGGTCAATAGGGGGTAATCAACAGGAAAGTCCCATTGGAGCCA
AGTACACTGCGTCAATAGGGACTTTCCATTGGGTTTGCCAGTACATAAGGTCAATAG
GGGATGAGTCAATGGGAAAACCCATTGGAGCCAAGTACACTGACTCAATAGGGACTTT
CCATTGGGTTTGCCTAAGTACATAAGGTCAATAGGGGGTGAGTCAACAGGAAAGTCCA
TTGGAGCCAAGTACATTGAGTCAATAGGGACTTCCAATGGGTTTGCCAGTACATAA
GGTCAATGGGAGGTAAGCCAATGGGTTTCCCATTACTGGCACGTACTGAGTCATT

Figure 14 (Continued)

AGGGACTTCCAATGGGTTTGCCTCAGTACATAAGGTCAATAGGGTGAATCAACAGGA
AAGTCCCATTGGAGCCAAGTACACTGAGTCAATAGGGACTTCCATTGGGTTTGCCTC
GTACAAAAGGTCAATAGGGGGTGAGTCATGGGTTTCCAGCCAATTAAATTAAAACGCCATGTAC
AGGTCAATAGGGGTGAGTCATTGGGTTTCCAGCCAATTAAATTAAAACGCCATGTAC
TTTCCCACCATTGACGTCAATGGGCTATTGAAACTAATGCAACGTGACCTTAAACGGT
ACTTTCCCAGCTGATTAATGGGAAAGTACCGTTCTCGAGCCAATACACGTCAATGGG
AAGTGAAGGGCAGCCAAACGTAACACCGCCCCGGTTTCCCTGGAAATTCCATATT
GGCACGCATTCTATTGGCTGAGCTCGTTCTACGTGGGTATAAGAGGGCGGACCAGCGT
CGGTACCGTGCAGTCTCGGTGACCACCGTAGAACGCAGAGCTCCTCGCTGCAGgc
ggccgcatgggctctaaacccattaccaggatcccagcacccatgtgtatcacc
gattatgtctgatattggactgtatccgtccgacaagctcttgcacggcaggccctttg
cagctgcaggaattgttagtaacaggagataaggcagtcaatgtatataccctcgatcag
acagggtcaatcatagtcagttgtcccaatatgcccaggataaggaggcgtgtgc
gaaagaccattagaggcatataacagaacactgactactttgtctactcctcttgcg
aatccatccgcaagatccaagggtctgtgtccacgtctggaggaggcaagcaaggccgc
ctgataggtgttattggtagtgttagctcttgggttgcaacagcggcacaataac
agcagctgcggccctaatacaagccaaccagaatgtgcacatcctcggcttaagg
agacattgtcaaccaatgaagctgtcatgaagtcaaccgcggattatcacaacta
tcaagtggcagttggaaagatgcacgcattgtcaatgaccagttataatacagcgc
agaattggactgtataaaaatcacacaacagggttgttagactcaacctatacctaa
ctgaatttgactacagtattcgggccacagatcacccctgcattaactcagctgacc
atccaggcactttataattagctgtggcaatatggattacttataactaagttagg
tatagggacaatacaactcagctcattaattggcagcggctgtatcactggttacccta
tattgtatgactcacagactcaactcttggcatacaagtgaatttgcctcagtcgg
aactaaataatatgcgtgccacctattagagacacctatctgttaagtacagccaaagg
atatgcctcagcacttgcataaaagttagtgcacacaagtgcgttctgtatagaagagc
ttgcacacccatactgtatagactccgatctggatttatattgtactagaatagtgaca
ttccccatgtccccaggattttattcctgtttaagcggcaacacatcagctgcattgt
ttcaaaagactgaaggcgcactcactacgcgtatatggccctaaaggctcagttattg
ccaattgtaaagataacaacatgttagatgtacagaccctcctggatcatatgc
aatatgtgagaagctgtatccctgtatagatagacattcgtcaatgtcttattcattagacgg
gataactctgaggctcagtgaggaaatttgcacacttataacttgcataacttgc
tagattctcaagtcatcgtgacaggcaatcttgcataacttgcataacttgc
aacaattcaatcagcaatgccttgataagttggaaaaagcaacagcaagctagaaaa
agtcaatgtcagactaaccagcacatccgcctcattacctatattgttgcactgtca
tttctctagtttgcgtgcactaagtctgggttaacatgttacctgtatgtacaaacaa
aaggcacaacaaaagacccattgtctatggctggataataccctcgatcagatgagagc
cactacaagagcatgagcggccgcgggatccagacatgataagatacattgtatgagtt

tggacaaaccacaactagaatgcagtgaaaaaatgtcttattgtgaaatttgcgtat
ctattgcattttgttaaccattataagctgcataataacaagttacaacaacaattgc
attgattttatgtttcaggttcagggggagggtgtggagggttttgcgtatcctctaga
gtcgacaaatttattttatataacatatagcacccaaagacccatgtatgcacatgtt
cccgatctcaacggcgcgtgtacacacgcacatctttgcatacgatgcataacttgc
cgacagcagaaaaatgcagatccaacaaatctggagaaaaacttgcatacactgc

Figure 14 (Continued)

tggaaacatacccccttatattcatggtataattatcgctcacagcgtccaggatagt
ggcgtgagaaaatggagatctgcagccctccttcatggcatgccgcctttattttca
ttaaacgcacaatggctcaacgccagatatggcatagattctgaagaaccgcgttgac
aatccgaagaagaaggcgtgcaggtctttggaagactcgcacgttggcttataatgtat
tgatcgagatgtcaccctaattgccacatggtacaggcttacgcggcatggcgatcgg
acttgtaatttgcacgatggcaaaggatcgacgacatgcacaaacattctgaaccgcgt
agagatgttaacgatgacgaggatgaatatcccattgcgtgcgtccatagtatcaagttac
accgcgaataaggacgcgtccaacatcgttatgcacacaatggctacacgcgtgacta
acaccccccgaatatttagtcatatgtgagttcagtcgtggctccatatacgctgttagac
tatttgtggttaagtgtgaacgaggcgcgtgtgaacgagactcggccgattgtaaagaa
caagcaaattgcactttccattnaacaagaagtgttagagagaatactcaacctttggat
tgtatcctcgag

Figure 14 (Continued)

Partial plasmid pCD046 + NDV Texas F for vHVT113 (SEQ ID NO:30)*Green and Italic = Flanking Arms*

BLUE AND UPPERCASE = mCMV IE

Black and Bold = NDV Texas FRed and underlined = SV40 Poly A

gagctcagggtatgatactcagctgttattgtggccgaccaggaggactccaatgc
tcattcataagaacgctagagatgctatttaacgatgtgctgtcgtaaaagaatttg
gcatttagccttaaatgtaaaaccaatgacgcattcactacgctcggtgcattt
ctgggccagggtatgcataattccataacagaaaatcgacacttgagaagaggatctgact
gtttggataaaggcgtttggctgtcgatataatttatgacgataata
ttaaacatctgtgtcgacttagttatcatgtcgatgaaatgttatgtgtaa
atatcggacaatatagataacgggcacgctgtattgttaacgtgcgcggcgctagt
gctgactaatagtgtggatgtatcacgtatattacaaacggaaatgatacgtata
aattatgtactcttattgatttataaaaacatacatgcagtgttgcacataat
tagcctcgcccgctacgctccactgaagataatggcgtcccgctgtcaaaaaatca
gcgtgcgtcgataagactttggcagtcgtctcgccgtcgcaatttagatttgc
tggagggtatctgggattttgccaatgctggagcgcacgtacgattcgccccat
cgggatctagcagaccaatgatgttgcacacatcgccatgcgtacggacggctca
ttgcgcgagttgttatttcgaaggacaagatggaagtgtatatggaccgacaataa
tgttagttgcatttcttagggcgaatctacatgatatacttgcataagcgggtatga
gccagagagatgtgtatggcataaaggtaatttttagatctgaaataacgcagttg
cccaaacaacgatcgcgattaaagaaaaatcgatggttcaattaggacatgcatt
ttctgtgcgcataaaaccataaccgcagcactgttggcacttcggtaactcaa
agcgttgcacgtctcgataactacgcctactatgcacattgttactcgtcatctt
aaatatatcctgttagtaattttcacagcaatgtcataacatcatctcgctaa
cctggattggagaagtaatgaatatttgcaccaatgcattgaataactacatt
acGAATTCAATAGTGGATCCCCAACTCCGCCGTTTATGACTAGAACCAATAGTTT
TAATGCCAATGCACTGAAATCCCCTAATTGCAAAGCCAAACGCCCTATGTGAGTA
ATACGGGGACTTTACCCAATTCCCACGCCGAAAGCCCCCTAATACACTCATATGGC
ATATGAATCAGCACGGTCATGCACCTCAATGGCGGCCATAGGGACTTCCACATAGGG
GGCGTTCACCATTCCAGCATAGGGTGGTGACTCAATGGCCTTACCCAAGTACATT
GGGTCAATGGGAGGTAAGCCAATGGTTTCCCATTACTGGCAAGCACACTGAGTCAA
ATGGGACTTCCACTGGTTTGCCAAGTACATTGGGTCAATGGGAGGTGAGCCAATG
GGAAAAACCCATTGCTGCCAAGTACACTGACTCAATAGGGACTTCCAATGGTTTTC
CATTGTTGGCAAGCATATAAGGTCAATGTGGGTGAGTCAATAGGGACTTCCATTGTAT
TCTGCCAGTACATAAGGTCAATAGGGGGTGAATCAACAGGAAAGTCCATTGGAGCCA
AGTACACTGCGTCAATAGGGACTTCCATTGGTTTGCCAGTACATAAGGTCAATAG
GGGATGAGTCAATGGGAAAACCCATTGGAGCCAAGTACACTGACTCAATAGGGACTTT
CCATTGGGTTTGCCAGTACATAAGGTCAATAGGGGGTGAATCAACAGGAAAGTTCCA
TTGGAGCCAAGTACATTGAGTCAATAGGGACTTCCAATGGTTTGCCAGTACATAA

Figure 14 (Continued)

GGTCAATGGGAGGTAAGCCAATGGTTTCCCATTACTGGCACGTATACTGAGTCATT
AGGGACTTCCAATGGTTTGCCCAGTACATAAGGTCAATAGGGTGAATCAACAGGA
AAGTCCCATTGGAGCCAAGTACACTGAGTCATAAGGGACTTCCATTGGTTTGCCCAGTACATA
AGGTCAATAGGGTGAAGTCATTGGTTTCCAGCCAATTAAATTAAAAGGCCATGTAC
TTTCCCACCATTGACGTCAATGGCTATTGAAACTAATGCAACGTGACCTTAAACGGT
ACTTTCCCATAGCTGATTAATGGGAAAGTACCGTTCTCGAGCCAATACACGTCAATGGG
AAAGTGAAAGGGCAGCCAAAACGTAACACCGCCCCGGTTTCCCCTGGAAATTCCATATT
GGCACGCATTCTATTGGCTGAGCTGCCTACGTGGGTATAAGAGGCGCACCAGCGT
CGGTACCGTGCAGTCTCGGTCTGACCACCGTAGAACAGCAGAGCTCCTCGCTGCAGGc
ggccgcatggctccagatcttaccagatcccgtacctctatatgctgatcatccg
aaccgcgctgacactgagctgtatccgtctgacaagctctttgatggcaggccttgc
cggctgcaggatcgtaacaggagataaagcagtcaacatatacacatcccag
acagggtcaatcatagttaagttactccgaatatgcccaggacaaagagggtgtgc
aaaagccccattggaggcatacacaaggacactgactactttactcaccccccttgg
attctatccgcaggatacaagagtctgtactactccggaggaggcaagcaaggccgc
ctgataggtgccattatccgcagtgttagcttgggttgcacagctgcacagataac
agcagttccgcctgatatacgccaaccagaatgctgccaacatcctccggcttaaag
agagcattgctgcaaccatgaagctgtgcacgaggactgacggattatcacaacta
gcagtggcagttaggaagatgcaacagttgtcaatgaccagttcaataatacagcgca
agaattggactgtataaaaattgcacagcaggctgttagaactcaacttgtaccaa
ctgaattgactacagtattggccacaatcactccctgccttaactcagctgact
atccaagcgcttacaatctagctggtaatatggattactgctgactaagttagg
tgttaggaacaaccaactcagctattaattggtagcggctgtacccggcaacccta
ttctgtacgactcacagactcagatcttgggtatacaggttaacttgcctttagtgg
aacctgaataatatgcgtgccacctacccggagaccttatctgttaagcacaaccaagg
atttgcctcagcacttgcctaaaaagtggtagacacaggctccgtatagaagaac
ttgacacccatactgtatagggaccgacttgattatactgtacaagaatagtgaca
ttccctatgtccctggatttattctgtctgagcggtaatacatccgcattgtcatgt
ttcaaagactgaaggcgcaactactacgcataatggctcaaaaggctcaggatttgc
ccaattgcaagctgacaacatgttagatgtcagatccccaggatcatatcgcaaaat
tatggagaagctgtgccttaatagataggcactcatgcaacgtcttaccccttagacgg
gataactctgaggctcagtggaaatttgcacccatcaaaaagaatatctctatac
tagattctcaagttatagtgcacaggcaatctgtatatactgagcttggaaatgtc
aacaactcaataagtaatgcctgaataagtttagggaaagcaacagcaaaactagacaa
agtcaatgtcaaaactgaccagcacatctgtctcattacccatcgttttaactgtca
tatctttgtttgtacttagcctgttctagcatgctacctgtatgtacaagcaa
aaggcacaacaaaagaccttggatggctggaaataatccctgtacatgagagc
cactacaaaatatgagccgcgggatccagacatgataagatacattgtatgt
tggacaaaccacaactagaatgcgtgaaaaaatgcattttgtgaaatttgtatgc
ctattgcattttgttaaccattataagctgcaataaaacaagttacaacaacaaattgc
attgatattatgtttcaggttcaggggaggtgtggaggttttccgtatgcatttgc
gtcgacaattatgttaataacatataggccaaagacctctatgaacatttagttt
cccgatactcaacggcgcgtgtacacacgcacatctttgcatacgatgtgaaatgttgc

Figure 14 (Continued)

cggcagcagaaaatgcagatatccaacaatctggagaaaacttatcatcacagtggcag
tggaaacatacccccttatattcatggtataattatcgctacagcgtccaggatagt
ggcgtgagaaaatggagatctgcagccctttccatggcatgccgcattattgtca
ttaaacgcacaatggtctcaacgccagatatggcatagattctgaagaaccgcgtgac
aatccgaagaagaaggcgtgcaggtcttggaaagactcgcacgttggtttataatgta
tgatcgagatgtcaccctaattgcccacatggtacaggcttatcgccgtcatggcgtatcg
acttgtaatttgcaacgatggcaaaggatcgacgacatgccaaacattctgaaccgcgt
agagatgttaacgatgacgaggatgaatatccatgctcgctgccatagtatcaagtac
accgcgaataaggacgcgtccaacatcgttatatgcacacaatggctacacgtgacta
acaccccgaaatattagtcatatgtgagttcagtcgtggctccatatacgctgttagac
tatttgtggtaagtgtgaacgaggcgctgtgaacgagactcggccgattgttaagaa
caagcaaatgcactttccathtaacaagaagtgttagagagaatactcaacctttgga
tgtatcctcgag

Figure 14 (Continued)

Partial plasmid pHM119 sequence for vHVT039 (SEQ ID NO:31)

Green and Italic = BamHI fragment I intergenic Recombination Arms

BLUE AND UPPERCASE = MDV gB PROMOTER

Black and Bold = NDV-F wild type unmodified Texas strain sequence

Red and Italic and Underlined = SV40 Poly A tail

gagctcagggtatgatactcagctgttattgtggccgaccaggaggactccaatgctta
gcattcataagaacgctagagatgctattaaacgtatgtctgtcgtaaaagaatttgt
gcatttagccttaatgtaaaaccaatgacgcattcactacgctcgctgcgtcaattt
ctgggccagggtatgcataattccataaacagaaaatcgacacttgagaagaggatctgact
gtttgggataaaaggcggtttgggtctgtccatgcgatataatttatgtacgatataaca
ttaaacatctgtgtgcagttacttaggtatttaatcatgtcgatgaaatgttatgtgtaa
atatcgacaaatatacgataacgggcacgatgttatgttacgtgcgcccgcgcgtatgt
gctgactaatagtgtggatgttatcacgttatattacaaacggaaatgatacgtaata
aattatgtactcttattgatttataaaaacatacatgcagtgttgcgtatgtcacataat
tagcctcgcccgctacgctccactgaagataatgggcgtcccgctgtcaaaaaaatca
gcgtgcgtcgataagactttggcagtcgtcttcgggtcgcaatttagattggccgca
tggagggtatctgggatttttgcataatgcgtggagcgacgtacgattcgccccat
cgggatctagcagaccaatgatgttgacacacatcgccatgcgtacggacggctta
ttgcgcgagttgttathttgcataaggacaagatggaaatgttatattggaaaccgacaataa
tgtagttgcatttcttagggcgaaatctacatgatatatccaaagcgggtatga
gccagagagatgtgtatggcataaaggtaaatttttagatctgaaataacgcagttg
cccaaacaacgatcgcgattaaagaaaaatcgatggttcaatttagacatgcgtatgg
ttctgtgcgcataaaccataaccgcagcactgttggcacttcggtactcaatgcga
agcgttgcacgtctcgatatactacgcctactatgcacattgttactcctgcattaa
aaatatatcctgtatgtatatttcacagcaatgtcataacatcatctcgatata
cctggattggagaagtaatgaatatttgcataaccatgcattgaataaactaacattaa
acgaattc**CGATGTTAGTCACGATAGACATCGGTCGCCAGCCGTCGAATACAGCAT**
TATATTAGTGTGAAAATGTAGGGCTGCTTCCTCACTTAAAGGAGGAAATGGCTCGA
TTCATGTTCATAGCAGTAGAAAAACAGATTGGACCGTCAGTAAGTTAGAGGGTTTA
TGACTTTAGCACTATAGATAATGTAACTGCAGGCCATCGCATGGCTGGAAATATATCA
AAGAACTGATTTGCAACAGCTTATTTCTGTATTTAAATGTGGCGAATTGCAC
ATCTGTCGTGCCGACAGTTGCAGATCAACAGCAATGGAGACTATGTATGGAAAAATGG
AATATATATAACATATGAAACCGAATATCCACTTATAATGATTCTGGGGTCAGAATCAA
GCACTTCAGAAACGCAAAATATGACTGCAATTATTGATACAGATGTTTTCTGCTT
TATTCTATTTGCACTATGGCCCCGTTACGGCAGATCAGGTGCGAGTAGAACAGAT
TACCAACAGCCACGCCCATCTGACCCGTCATAATTCTGTGTCCTGCATTTC
TCACACAATTGAAACAGCATCTAACAGATCATCTCACTgcggccgcaag**atgggctc**
cagatcttctaccaggatcccggtacctctaattgtatcatccgaaccgcgcgtacac
tgagctgtatccgtctgacaagctctttgtatggcaggcctttgcggctgcaggatc

Figure 14 (Continued)

gtggtaacaggagataaagcagtcaacatatacacacctcatcccagacagggtcaatcat
agttaagttaactcccaaatatgcccaggacaaagagggtgtgtcaaaaagccccattgg
aggcataacaacaggacactgactactttactcaccccccttggtagtttatccgcagg
atacaagagtctgtgactacttccggaggaaggagacagagacgccttatagggccat
tatcgccagtgtagcttgggttgcacagctgcacagataacagcagcttggccc
tgatacaagccaaccagaatgctgccaacatcctccggcttaaagagagacattgctgca
accaatgaagctgtgcacgaggtcactgacggattatcacaacttagcagtggcagtagg
gaagatgcaacagttgtcaatgaccagttcaataatacagcgcaagaattggactgt
taaaaattgcacagcaggtcggttagaactcaacttgtacctaactgaattgactaca
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aactcagctcattaattggtagcggcttgcacccctatttgtacgactca
cagactcagatcttgggtatacaggttaacttgccttcagttggaaacctgaataat
gcgtgccacacctggagacccatctgtgtaagcacaaccaagggatttgcctcagcac
ttgtcccaaagtgtgacacaggtcggttgcataagaacttgacacccctac
tgtatagggaccgacttggatttatactgtacaagaatagtgacattccatatgtctcc
tggatttattctgtctgagcggtaatacatcggttgcattgtattcaaagactgaag
gcgcacttactacgcccataatggctctcaaaggctcagttattgccaattgcaagctg
acaacatgttagatgtcagatccccaggtatcatatcgcaaaattatggagaagctgt
gtccttaatagataggcactcatgcaacgtcttaccccttagacgggataactctgaggc
tcagtgggaatttgatgcaacctatcaaaaagaatatctctatacttagattctcaagtt
atagtgcacggcaatcttgatatatcaactgagcttggaaatgtcaacaactcaataag
taatgcctgaataagtttagagggaaagcaacagcaaaacttagacaaaagtcaatgtcaaac
tgaccacatctgcttcattacacctacatcggttactgtcatatcttttt
ggtgtacttagcctgggtctagcatgctacctgatgtacaagcaaaaggcacaacaaa
gaccttggtatggcttggaaataatcccttgcatacagatgagagccactacaaaaat
gacggccgcgggatccagacatgataagatacattgtatggatgtttggacaaaccacaa
ctagaatgcagtgaaaaatgcattttttgtgaaatttgcattgtattgcatttattt
gtaccattataagctcaataaacaagttacaacaacaattgcattcattttatgtt
tcaggttcaggggagggtgtggagggttttcggatcctctagagtgcacaatttattt
tatttaataacatataggccaaagaccttatgcataacattttagttccctgtataactcaac
ggcgcgtgtacacacgcattttgcatacgatgaaagttgttgcggcagcagaaaat
gcagatataccaaatctggagaaaacttatcatcacagtggcagtgagaaacatacc
ctctatattcatggtataattatcgctacagcgtccaggatagtggcgtgagaaaatg
gagatctgcagccctcatttgcattggcattttgcgttgcggcagcacaat
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ccctaattgcacatggtacaggcttatcgccgtcatggcgtatggacttgcatttgc
acgatgggcaaggatcgacgcacatgccaacacattctgaaaccgttagagatgttacga
tgacgaggatgaaatattccatgcgtcgatgtatcaagtacaccgcgaataagga
cgcgtccaacatcgatgttatgcacacaatggctacacgtgactaacaaccccccgaat
tagtcataatgtgagttcagtcgtccatatacgctgttagactattgtggttaa
gtgtgaacggcgtgtgaacgagactcggccgattgtaaagaacaagcaatgcact
ttccatattaacaagaagtgttagagagaatactcaaccttggatgtatcctcgag

Figure 14 (Continued)

Partial plasmid SORF3-US2 gpVar-Ewtsyn sequence (for vHVT202) (SEQ ID NO:39)*Green and Italic = Flanking Arms***BLUE AND UPPERCASE = GPCMV****Black and Bold = Variant E wt***Red and Italic and Underlined = Syn Poly A*

taaaaatggatctatcattacattcgtaagagtctggataattttactgtttgccagc
ttcgatcttggAACGTACTGTGGATAGTGCCTACTTGGAAATCGTGAaaaATTGAAACG
tccattatttggatatcttccggttgtcccatatcccgcctggTACCGCTCGATAcc
ttgcccgtatggattcgtattgacagtgcgcAATCGGGGACCAACAACGCGTgggtcc
acactcattcgaaatttccgatgattctgaatatttatttgcgtcgatcgagtcg
ttggacatataCTGTAatacatttcttctgaaggatcgctgcacatttgcata
attggccaggatgtcaagtctcagatgttgcatttgcacagcacaactttatggca
tttccgatgtaatcgccggcagccctggggagttctataattcgcatattggatgg
aaggacaatagcagatctcgcaacctccaggaggctataataacgttttaaaggatg
gatttctcataaaaatctgtcgcAAttacactgagaataatccttactagcggcgatt
gagagcatcgctgtccaaatttctaaatggaaagaaaacaaggcggcaagagtgtcc
aaacattttcatttcggcgaatctctcaaattccatggcgtcaattgattgaaaaat
tggcacttccgttacgttgtatctccaaactctaagacacttttaattgaaaaacta
cgttctagtgtggaaagaaaacctataggcagaccatagaactattgacaccatata
ttttgtatgtcaactgaccatgatcgatgttgcattgcactaggcaattcgct
cgccgcactccatacattgaataattccacacgtcagctcatcggttagcaaggtccag
tagttgaagtcatttatTTTCCCGCgctgtggcatggtaaggaaacttgcataaaagac
gcaggtatcatagggtaatatTTTTTATTcactcacataactaaaagtaacgcata
agcaccatgtatggctatcaattgacatttgcgttagcactacatcaccatgtaca
acataatggacaacatatgcctgcaggTTAGTCATATGTTACTGGCAGAGGCCGCAT
GGAAAGTCCCTGGACGTGGACATCTGATTAATACGTGAGGAGGTAGCCATGTTCTT
TTGGCAAAGGACTACGGTATTGGACGTTGATTGGCATGGGATAGGGTCAGCCAGAGT
TAACAGTGTCTTTGGCAAAGGGATACGTGGAAAGTCCCAGGCCATTACAGTAAACT
GATACGGGGACAAAGCACAGCCATATTAGTCATGTATTGCTTGGCAGAGGGCTATGG
AAAGTCCCTGGACGTGGACGTCTGATTAATATGAAAGAAGGTAGCCAGAGGTAGCTG
TGTCTTTGGCAAAGGGATACGGTTATGGACGTTGATTGGACTGGGATAGGGTCA
GCCAGAGTTAACAGTGTCTTTGGCAAAGGAACGTGGAAGTCCCAGGCCATTACAG
GTAAACTGATACTGGACAAAGTACACCCATATTAGTCATGTATTGCTTGGCAAAGAG
CATCTGGAAAGTCCCAGGCCAGCATTATAGTCATGGCAGAGGGAAAGGGTCACTCAGA
GTTAAGTACATCTTCAGGGCAATATTCCAGTAAATTACACTTAGTTTATGCAAAT
CAGCCACAAAGGGATTTCGGGTCAATTATGACTTTCTTAGTCATGCGGTATCC
AATTACTGCCAAATTGGCAGTACATACTAGGTGATTCACTGACATTGGCAGTCTG
GAAAGTCCCTGGAAACCGCTCAAGTACTGTATCATGGTGACTTGCATTGGAGAGC
ACGCCCACTCCACCATTGGTCCACGTACCCCTATGGGGAGTGGTTATGAGTATATAA
GGGGCTCCGGTTAGAACGCCAGAGCGGCGCAGAGCAGGCGCAGAGCAGGCGCAGAGC
atgacaaaacctgcataagatcaaacc

Figure 14 (Continued)

caacagattgtccgttatacgagcctctgatgccaacaaccggaccggcgccat
tccggacgacaccctggagaaggcacactctcaggtcagagacacctgacataattga
ctgtggggacacagggtcagggctaattgtctttccctggattccctggctcaatt
gtgggtgctcactacacactgcagagcaatggaaactacaagttcgatcagatgtcct
gactgcccagaacacctaccggccagctacaactactgcaggctagttagtcggagtc
cagtaaggtcaagcacactccctggcggttatgcactaaacggcaccataaacgcc
gtgaccttccaaggaaggcctgagtgaaactgacagatgttagtacaacgggttatgtc
tgcaacagccaaacatcaacgacaaaattggaaacgtcctagtagggaaagggtaaccg
tcctcagcttacccacatcatatgatcttgggtatgtgaggcttgcacccatacc
gctataggcgttgcacccaaaaatggtagcaacatgtgacagcagtgcacaggcccag
ctacaccataactgcagccgataattaccaatttcattcacactaccatcacagggtgggg
taacaatcacactgttctcagccaaacattgtatgcattcacaatgtctcagcgttgggg
gagctcgttcaaaacaagcgtccaaaggcttgtactggcgccaccatctacatt
aggctttagtggactgcggtaatcaccagagctgtggccgaaacaatggctgacgg
ccggcatcgacaatcttattgcattcaatcttgcatttgcattcaacatgagataacccag
ccaatcacatccatcaaactggagatagtgcacccaaaagtgtatggcaggcagg
acagatgtcatggcgcaagtggagcctagcagtgcacatggcaactatc
caggagccctccgtcccgtaactactgtgcctacgaaagagtggcaacaggatctgc
gttacggcgttgcgtgggttagcaacttcgcagctgatcccaatcttgcattcaac
cctggttacagaatatggcgatttgaccaggccatgaactacacgaaattgata
tgagttagggaccgccttgcattcaagaccgtctggcaacaaggagactacgtac
ttcgttagtacttcattggaggtggccgacactctccctgaagattgcaggagc
atttggctcaaagacataatccggccataaggaggtgagcggccgcataataa
aatatcttatttcattacatctgtgtttttgtgtgaatcgatagtactaa
catacgctctccatcaaaacaaaacgaaacaaaactagcaaaataggctgtcccc
agtgcacatgtgcagggtgcacacatttcttcttagacctgcaggccggcaagtag
atgcacatttcctcacactagttgggttatctactattgaattttccctatctgtat
acacttgggagccctacaagcatattgcattcatgtacgttttatctactgtctta
cgcccatgggaacggaggcgtcgtcatgtattggacggcaacataggcaggcaacac
aaattgcgttaggtgggtgcattgtggactcgataccacgcggccatattactgtc
gtctggtagggccatatttgatatacgccatattactgtcgttgaagtac
ccttatcttctatgtttcaatatttaggtcccaagtggacgtgagaagtgttgtatc
tcacatggaaatggcccaaggcatccagcccagggtgcctggactttaatggca
acgttttgttaggatattgatttcattgcagttctgcagatctgcagcccgagta
tccacaggctatacgatccatgttgcggaggcctccgattctgcattacatagccg
agtagatcctgcattcggtagcgcacccggctacatcttcaaacagtcataataa
tgcatctctcggttgcattccgcataatccggaaaccggccataccactccgc
ttctcacaattggcgatgcggcgccggcaaaacgaatgtggatttgcaaaaccg
acaggctgcgttgcggactaatatgggcacacccacatcattcttcagatgc
ttgttctatgagaaaagatccatagggtggaggcaggcgtcagagatgc
gatgcgcattcgtcttagttaaagtgcacgcgttgcacacacccatgc
tccgaataactggagctgtggaaagatcgaaaacgtcttttgc
actttcgcacagggtgtatacccgacgcgtactatatatttatcatcc
gaaattacatacggtggcgatggaaagtagatgttgc
gaaatgtgcctc

Figure 14 (Continued)

gaatatgggtattgtctgtgaaaatatcgaaagcggtaacgacggttgcagaaccgtcga
tgtccgcagatacttagtaacaatagcttcgataacgaagacttccgtggcctgaatac
gatgtggagata

Figure 14 (Continued)

Partial plasmid SB1US2 gpVIIIdwtsyn sequence (for vSB1-010) (SEQ ID NO:40)*Green and Italic = Flanking Arms**BLUE AND UPPERCASE = GPCMV***Black and Bold = NDV-F VIIId wt***Red and Italic and Underlined = Syn Poly A*

tctcgtctaaaacgctccagtgcttacagttcgataatctggacctggggacgcgtat
aggatcggttcctccacatgcgctgctgtcggtatctcgaatccccggattcagttgaa
tcgttggcggagtgtcctcctggactctgcaatgttccctagccgtcttactatctcg
tgcaaggctctataatacagttcctctgcagacccgtcggtcttcccttgcgtc
gttagttatttctgttaggctccagacgattgcctgcatttgtgcgcaacataatctga
ttgcattccatatctcgcttccggtaatccatagggtttcggtattcgcagatagg
agagaaaagcaccactgcaaatctgtcaatttccattgccccaaaccaatattttttaa
gaacggcatcgccgttaatgtacacctcggcattgtgacgatcgaaacccttatggatgc
ctaaagagagcattgcgggccagttctccaggtgaaaaagagaatagcgcgggtagaaaac
ggccgatttagtttatcttcggccgtccctaataatcccaagttctgcagtataactt
ccatcgtccgttttcgacaagggtccggcgcacatagttgaaatgtcatctatcagaa
acatctcgccatcgtagaaaaaacctgtacgcagaccataaaaccattcggtaccac
atatccttgttatatcaaacgatatgttggttatgtcggttggcgatgtgtatgaaa
tagagctaagcggtctggattccacgcactgaacgattccgttagtcaattcatctg
ctaacataggccaaaagttattcgtgttactttctcggcggttggcaaaacgcccc
cttggcacatccatgtcattaaatacagcggcataactcctactcatgtgttccatagc
ccaggttcttcggctgctgactacgatcagatcgtggcgcgatcagatgcgtgg
atgaatgaagtgtatccgaaagcagttttagatatacgctaaactgtacgcacgattgt
ggcactaaacgaagcttgcgcaccccccattccacgcgcctgcaggTTAGTCATATGTT
ACTTGGCAGAGGCCGCATGGAAAGTCCCTGGACGTGGACATCTGATTAATACGTGAGG
AGGTCAAGCCATGTTCTTTGGCAAAGGACTACGGTCATTGGACGTTGATTGGCATGG
GATAGGGTCAGCCAGAGTTAACAGTGTCTTGGCAAAGGGATACTGGAAAGTCCCG
GGCCATTACAGTAAACTGATACTGGGGACAAAGCACAGCCATATTAGTCATGTATTGC
TTGGCAGAGGGTCTATGGAAAGTCCCTGGACGTGGACGTCTGATTAATATGAAAGAAG
GTCAGCCAGAGGTAGCTGTGCTTTGGCAAAGGGATACTGGTATGGGACGTTGAT
TGGACTGGATAGGGTCAGCCAGAGTTAACAGTGTCTTGGCAAAGGGAAACGTGGAA
AGTCCCAGGGCATTACAGTAAACTGATACTGGGACAAAGTACACCCATATTAGTCAT
GTTCTTTGGCAAAGAGCATCTGGAAAGTCCGGCAGCATTATAGTCACTTGGCAGA
GGGAAAGGGTCACTCAGAGTTAAGTACATCTTCCAGGGCAAATATTCCAGTAAATTAC
ACTTAGTTATGCAAATCAGCCACAAAGGGATTTCGGCAGTACATACTAGGTGATTCACTG
CTTAGTCATGCGGTATCCAATTACTGCCAAATTGGCAGTACATACTAGGTGATTCACTG
ACATTGGCCGTCCTCTGGAAAGTCCCTGGAAACCGCTCAAGTACTGTATCATGGTGAC
TTTGCATTTGGAGAGCAGCCCCACTCCACCATTGGTCCACGTACCCATGGGGAG
TGGTTATGAGTATATAAGGGGCTCCGGTTAGAACGCCGGCAGAgcggccgcatgggc
tccaaacccatcaccaggatcccagcaccctctgtatgtatcaccgcattatgtat
attggcgtgtatccgtccgacaaagctctttgacggcaggccttgcagctgcaggaa
ttgttagtaacaggagataaggcagtcataacttcgtctcagacagggtcaatc

Figure 14 (Continued)

atagtcaagttgctcccgaatatgcccaggataaggaggcggtgc
 agaggcatataacagaacactgactactttgctactccttgcgcactccatccgca
 agatccaagggtctgttccacatctggaggaggcaagcaaggccgcctgataggtgct
 gttattggcagtgttagctcttgggggttgcaacacagcggcacagataacacagcagctgcggc
 cctaataacaagccaaccagaatgccgccaacatcctccggcttaaggagagcattgctg
 caaccatgaagctgtcatgaagtaccgcggatttatcacaactatcagttgcaggatt
 gggaaagatgcagcagttgtcaatgaccatTTTAAATAACGGCGCAGAATTGGACTG
 tataaaaaatcacacacaacaggttgggttagaactcaacctatacctaactgaatttgacta
 cagtattcggggcacagatcacccctgcatttaactcagtcgaccatccaggcactt
 tataatttagctgggtggcaatatggattacttattaactaaggtaggtatagggaaacaa
 tcaactcagctcgtaattggtagcggcctgatcactggttaccctatactgtatgact
 cacagactcaactcttgggcatacaactgaatttaccctcagtcgggaaacttaataat
 atgcgtgccacactattggagacaccttatctgttaagtacaaccaaaaggatatgcctc
 acttgcggcaaaagttagttagcacaactgcgggtccgtatagaagagacttgcacac
 actgtatagagtccgatctggatttatattgtactagaatagttagtgcacattccatgtcc
 ccaggtatttattccgtttgagcgcaacacatcagctgcattgcatttcaaagactga
 aggccactcaactacgcccgtatatggccctttaaaggctcagttattgccaattgtaaaa
 taacaacatgttagatgtacagacccctctggtatcatatcgaaaaattatggagaagct
 gtatccctgatagatagacattcgtcaatgtcttattcattagacggataactctaag
 gctcagtgaaaatttgatgcaacttatcaaaagaacatctcaataacttagattctcaag
 tcatcgtgacaggcaatcttgcattatcaactgaacttggaaacgtcaacaattcaatc
 agcaatgccttggatagttggcagaaagcaacacagcaagctagaaaaagtcaatgtcag
 actaaccagcacatctgctcattacctatattgttctacttgtcattcttagttagt
 tcgggtcacttagtctggtagcgtgttacctgatgtacaaacagaaggcacaacaa
 aagaccttgcataggcttgggaaaataataccctcgatcagatgagagccactacaagagc
atgagcgccgcgatataaaaatatctttatccattacatctgtgtttgg
tttgtgtgaatcgatagactaacatacgcctccatcaaaaacaaaacgaaacaaaaca
aactagaaaaataggctgtccccagtgcaagtgcaggtgccagaacatccatctctct
acctgcaggggagttgtgcaggtaatgaccctcgcagttcattcggaaagttataac
tgccgccttcgcacattttttgtcctgtttgtattgccataacagataggaatttg
aaacctgatcctctgttttgacatggccaggcaacagaataactttgtcgatcg
ctacttgcgcgagatgggtccgttctggagggtttcggcgggtcggtggagaacactat
tatTTTatacacacacgtcataccgttgcgaaaatgttcttgcattctgcgtct
cgaacgtcggtccacgttagacgttagggcgttggatgttatcaggaagagccccac
ggcatgcccggaccaagtaaccgcgtactttgaccgcgagcagtcttgcgtatggat
gtattccagagcagcgcggcagagatcagcggccccactatccacacactgttatgaag
tgtttctgtaaacatcgactccaacatcaaataccagacatacatcttgcattcg
gaagcacatccgcgcacatcttcaaatagcctaactataaacagagtctctagttc
taaccaggactcgaatgccagtcggccatccgggtgggtcgatcatcggtctct
gacgcgcggagaaactaaaagggtctggaaaagcggaacagatctgcagaccgaac
actacacacacgcccacatcatcatgtatctgttccatgcattgcatttgcatttg
ccataaggccgaggcggcatctctagatctccggggagttctcgactcatcttagga
gagtgcacagttatcatagacacgcggcattttgtgcaccaaaacgaaaagttc
ctgttgcacaccatcgatgttttgatcgaaaccagtgtctagacagaaga
tggtgagcgtcggcggatcggtccgtgcattgtgtgtctgcacaccatcgatgt

Figure 14 (Continued)

*ccatccggtaaaattctggtgtatgaactgacggtctccagacgaaacgtcgaagacatta
acgatggaaaactaacgagcttcattcaaaagtgtctgattacaacgctaatacgacctta
cgaaactatacgcagcgataccagtgacacagatccgtcggtgtcg*

1

**RECOMBINANT HVT VECTORS
EXPRESSING ANTIGENS OF AVIAN
PATHOGENS AND USES THEREOF**

**CROSS-REFERENCE TO RELATED
APPLICATIONS**

This application claims priority to U.S. provisional application 61/564,877 filed on Nov. 30, 2011 and U.S. provisional application 61/694,957 filed on Aug. 30, 2012.

FIELD OF THE INVENTION

The invention relates to recombinant viral vectors for the insertion and expression of foreign genes for use as safe immunization vehicles to protect against a variety of pathogens. It also relates to multivalent composition or vaccine comprising one or more recombinant viral vectors for protection against a variety of pathogens. The present invention relates to methods of making and using the recombinant viral vectors.

BACKGROUND OF THE INVENTION

Poultry vaccination is widely used to protect poultry flocks against devastating diseases including Newcastle disease (ND), infectious bursal disease (IBD), Marek's disease (MD), infectious bronchitis (IB), infectious laryngotracheitis (ILT) and avian influenza (AI). ND is caused by the avian paramyxovirus 1 (APMV-1) also designated ND virus (NDV) belonging to the Paramyxoviridae family. MD is caused by Gallid herpesvirus 2 (Herpesviridae family) also designated as MD virus serotype 1 (MDV1). IB is caused by IB virus (IBV) belonging to the Coronaviridae family, ILT is caused by Gallid herpesvirus 1 (Herpesviridae family) also designated ILT virus (ILTV) and AI is caused by AI virus (AIV) belonging to the Orthomyxoviridae family.

A number of recombinant avian viral vectors have been proposed with a view to vaccinating birds against these avian pathogens. The viral vectors used comprise avipox viruses, especially fowlpox (EP-A-0,517,292), Marek's virus, such as serotypes 2 and 3 (HVT) (WO-A-87/04463), or alternatively the ILTV, NDV and avian adenovirus. When some of these recombinant avian viral vectors were used for vaccination, they display variable levels of protection.

Several recombinant herpesvirus of turkeys (HVT, also designated Meleagrid herpesvirus 1 or MDV serotype 3) vectors expressing antigens from various pathogens (U.S. Pat. Nos. 5,980,906, 5,853,733, 6,183,753, 5,187,087) including IBDV, NDV, ILTV and AIV have been developed and licensed. Of particular interest is a HVT vector-expressing IBDV VP2 protective gene that has shown clear advantages over classical IBD vaccines (Bublot et al J. Comp. Path. 2007, Vol. 137, S81-S84; U.S. Pat. No. 5,980,906). Other HVT vectors of interest are those expressing either NDV (Morgan et al 1992, Avian dis. 36, 858-70; U.S. Pat. No. 6,866,852; U.S. Pat. No. 5,650,153) or ILTV (Johnson et al, 2010 Avian Dis 54, 1251-1259; U.S. Pat. No. 6,299,882; U.S. Pat. No. 5,853,733) protective gene(s). One of the practical problems of using several HVT-based recombinant vaccines together is their interference. Lower protection is induced at least against one of the disease when two HVT recombinants expressing different antigens are mixed (Rudolf Heine 2011; Issues of the Poultry Recombinant Viral Vector Vaccines which May Cause an Effect on the Economic Benefits of those Vaccines; paper presented at the XVII World Veterinary Poultry Association (WVPA) Congress in Cancun, Mexico, Aug. 14-18,

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2011; Slacum G, Hein R. and Lynch P., 2009, The compatibility of HVT recombinants with other Marek's disease vaccines, 58th Western Poultry Disease Conference, Sacramento, Calif., USA, March 23rd-25th, p 84).

5 The combination of HVT and SB-1, a Gallid herpesvirus 3 (MDV serotype 2 or MDV-2) vaccine strain, has shown a synergistic effect on MD protection (Witter and Lee, 1984, Avian Pathology 13, 75-92). To address the interference problem, it is of interest to evaluate the HVT virus as a vaccine 10 vector to express one or more protective antigen(s) against a variety of avian pathogens.

The SB-1 genome was cloned and characterized in bacterial artificial chromosome (BAC) (Petherbridge, et al., J. Virol. Methods 158, 11-17, 2009; Singh et al., Research in 15 Veterinary Science 89, 140-145, 2010). The MDV2 SB-1 sequence was recently obtained and analyzed (Spatz and Schat, Virus Gene 42, 331-338, 2011). A glycoprotein E deletion of SB-1 virus was described by Petherbridge, et al. (J. Virol. Methods 158, 11-17, 2009). However, no research 20 has been reported using SB-1 as a viral vector expressing foreign protective genes.

Considering the potential effect of animal pathogens, such as NDV and IBDV on veterinary public health and the economy, efficient methods of preventing infection and protecting animals are needed. There is a need for a solution of combined effective vector vaccines and a suitable method for making the vaccine that could alleviate the problem of interference observed between two HVT-based vector vaccines.

SUMMARY OF THE INVENTION

The present invention showed surprising result when polyvalent compositions or vaccines comprising single or double HVT vector were effective to protect animals against a variety of avian pathogens without interference. Surprising results 35 were also observed when various combinations of promoters, codon-optimized gene, polyA tails and insertion sites conferred different levels of efficacy and stability to the expression of one or more heterologous genes in vivo.

40 The present invention relates to a recombinant HVT vector comprising one or more heterologous polynucleotides coding for and expressing at least one antigen of an avian pathogen.

The present invention provides a composition or vaccine comprising one or more recombinant HVT vectors comprising 45 one or more heterologous polynucleotides coding for and expressing at least one antigen of an avian pathogen.

The present invention provides a polyvalent composition or vaccine comprising one or more recombinant HVT vectors comprising heterologous polynucleotides coding for and expressing 50 at least one antigen of an avian pathogen and one or more recombinant SB1 vectors comprising heterologous polynucleotides coding for and expressing at least one antigen of an avian pathogen.

The present invention relates to a method of vaccinating an 55 animal, or inducing an immunogenic or protective response in an animal, comprising at least one administration of the composition or vector of the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

60 The following detailed description, given by way of example, and which is not intended to limit the invention to specific embodiments described, may be understood in conjunction with the accompanying figures, incorporated herein by reference, in which:

65 FIG. 1 is a table showing the SEQ ID NO assigned to each DNA and protein sequence.

FIG. 2 depicts the genome structure of HVT and its insertion sites.

FIG. 3 depicts the plasmid map of pHM103.

FIG. 4 depicts the PCR analysis results of vHVT114.

FIG. 5 shows the dual immunofluorescent assay results. FIG. 5A1 and FIG. 5A2 are from the pre-MSV passage. FIG. 5B1 and FIG. 5B2 are from the pre-MSV+12 passage.

FIG. 6 depicts the Southern blot results of vHVT114.

FIG. 7 depicts the immunoprecipitation and Western blot analysis results of vHVT114.

FIG. 8 depicts the Western blot analysis of immunoprecipitated sample from vHVT306 infected cells.

FIG. 9 depicts the Western blot analysis of immunoprecipitated sample from vSB1-009 infected cells.

FIG. 10 depicts the result of challenge study of vHVT304 and vHVT114 against NDV ZJ1 and CA02.

FIG. 11 depicts the viral shedding result after NDV CA02 and ZJ1 challenge. FIG. 11A depicts the vial shedding result after CA/02 challenge. FIG. 11B depicts the vial shedding result after ZJ1 challenge.

FIG. 12A and FIG. 12B depict the viral shedding result after NDV Chimalhuacan challenge.

FIG. 13 shows the sequence alignment and percentage identity.

FIG. 14 shows the DNA and protein sequences.

DETAILED DESCRIPTION OF THE INVENTION

It is noted that in this disclosure and particularly in the claims, terms such as "comprises", "comprised", "comprising" and the like can have the meaning attributed to it in U.S. Patent law; e.g., they can mean "includes", "included", "including", and the like; and that terms such as "consisting essentially of" and "consists essentially of" have the meaning ascribed to them in U.S. patent law, e.g., they allow for elements not explicitly recited, but exclude elements that are found in the prior art or that affect a basic or novel characteristic of the invention.

Unless otherwise noted, technical terms are used according to conventional usage. Definitions of common terms in molecular biology may be found in Benjamin Lewin, *Genes V*, published by Oxford University Press, 1994 (ISBN 0-19-854287-9); Kendrew et al. (eds.), *The Encyclopedia of Molecular Biology*, published by Blackwell Science Ltd., 1994 (ISBN 0-632-02182-9); and Robert A. Meyers (ed.), *Molecular Biology and Biotechnology: a Comprehensive Desk Reference*, published by VCH Publishers, Inc., 1995 (ISBN 1-56081-569-8).

The singular terms "a," "an," and "the" include plural referents unless context clearly indicates otherwise. Similarly, the word "or" is intended to include "and" unless the context clearly indicate otherwise. The word "or" means any one member of a particular list and also includes any combination of members of that list.

The term "animal" is used herein to include all mammals, birds and fish. The animal as used herein may be selected from the group consisting of equine (e.g., horse), canine (e.g., dogs, wolves, foxes, coyotes, jackals), feline (e.g., lions, tigers, domestic cats, wild cats, other big cats, and other felines including cheetahs and lynx), bovine (e.g., cattle), swine (e.g., pig), ovine (e.g., sheep, goats, lamas, bison), avian (e.g., chicken, duck, goose, turkey, quail, pheasant, parrot, finches, hawk, crow, ostrich, emu and cassowary), primate (e.g., prosimian, tarsier, monkey, gibbon, ape), humans, and fish. The term "animal" also includes an individual animal in all stages of development, including embryonic and fetal stages.

The terms "polypeptide" and "protein" are used interchangeably herein to refer to a polymer of consecutive amino acid residues.

The term "nucleic acid", "nucleotide", and "polynucleotide" are used interchangeably and refer to RNA, DNA, cDNA, or cRNA and derivatives thereof, such as those containing modified backbones. It should be appreciated that the invention provides polynucleotides comprising sequences complementary to those described herein. The "polynucleotide" contemplated in the present invention includes both the forward strand (5' to 3') and reverse complementary strand (3' to 5'). Polynucleotides according to the invention can be prepared in different ways (e.g. by chemical synthesis, by gene cloning etc.) and can take various forms (e.g. linear or branched, single or double stranded, or a hybrid thereof, primers, probes etc.).

The term "genomic DNA" or "genome" is used interchangeably and refers to the heritable genetic information of a host organism. The genomic DNA comprises the DNA of the nucleus (also referred to as chromosomal DNA) but also the DNA of the plastids (e.g., chloroplasts) and other cellular organelles (e.g., mitochondria). The genomic DNA or genome contemplated in the present invention also refers to the RNA of a virus. The RNA may be a positive strand or a negative strand RNA. The term "genomic DNA" contemplated in the present invention includes the genomic DNA containing sequences complementary to those described herein. The term "genomic DNA" also refers to messenger RNA (mRNA), complementary DNA (cDNA), and complementary RNA (cRNA).

The term "gene" is used broadly to refer to any segment of polynucleotide associated with a biological function. Thus, genes or polynucleotides include introns and exons as in genomic sequence, or just the coding sequences as in cDNAs, such as an open reading frame (ORF), starting from the start codon (methionine codon) and ending with a termination signal (stop codon). Genes and polynucleotides can also include regions that regulate their expression, such as transcription initiation, translation and transcription termination. Thus, also included are promoters and ribosome binding regions (in general these regulatory elements lie approximately between 60 and 250 nucleotides upstream of the start codon of the coding sequence or gene; Doree S M et al.; Pandher K et al.; Chung J Y et al.), transcription terminators (in general the terminator is located within approximately 50 nucleotides downstream of the stop codon of the coding sequence or gene; Ward C K et al.). Gene or polynucleotide also refers to a nucleic acid fragment that expresses mRNA or functional RNA, or encodes a specific protein, and which includes regulatory sequences.

The term "heterologous DNA" as used herein refers to the DNA derived from a different organism, such as a different cell type or a different species from the recipient. The term also refers a DNA or fragment thereof on the same genome of the host DNA wherein the heterologous DNA is inserted into a region of the genome which is different from its original location.

As used herein, the term "antigen" or "immunogen" means a substance that induces a specific immune response in a host animal. The antigen may comprise a whole organism, killed, attenuated or live; a subunit or portion of an organism; a recombinant vector containing an insert with immunogenic properties; a piece or fragment of DNA capable of inducing an immune response upon presentation to a host animal; a polypeptide, an epitope, a hapten, or any combination thereof. Alternately, the immunogen or antigen may comprise a toxin or antitoxin.

The term "immunogenic protein or peptide" as used herein includes polypeptides that are immunologically active in the sense that once administered to the host, it is able to evoke an immune response of the humoral and/or cellular type directed against the protein. Preferably the protein fragment is such that it has substantially the same immunological activity as the total protein. Thus, a protein fragment according to the invention comprises or consists essentially of or consists of at least one epitope or antigenic determinant. An "immunogenic" protein or polypeptide, as used herein, includes the full-length sequence of the protein, analogs thereof, or immunogenic fragments thereof. By "immunogenic fragment" is meant a fragment of a protein which includes one or more epitopes and thus elicits the immunological response described above. Such fragments can be identified using any number of epitope mapping techniques, well known in the art. For example, linear epitopes may be determined by e.g., concurrently synthesizing large numbers of peptides on solid supports, the peptides corresponding to portions of the protein molecule, and reacting the peptides with antibodies while the peptides are still attached to the supports. Similarly, conformational epitopes are readily identified by determining spatial conformation of amino acids such as by, e.g., x-ray crystallography and 2-dimensional nuclear magnetic resonance.

The term "immunogenic protein or peptide" further contemplates deletions, additions and substitutions to the sequence, so long as the polypeptide functions to produce an immunological response as defined herein. The term "conservative variation" denotes the replacement of an amino acid residue by another biologically similar residue, or the replacement of a nucleotide in a nucleic acid sequence such that the encoded amino acid residue does not change or is another biologically similar residue. In this regard, particularly preferred substitutions will generally be conservative in nature, i.e., those substitutions that take place within a family of amino acids. For example, amino acids are generally divided into four families: (1) acidic—aspartate and glutamate; (2) basic—lysine, arginine, histidine; (3) non-polar—alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan; and (4) uncharged polar—glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine. Phenylalanine, tryptophan, and tyrosine are sometimes classified as aromatic amino acids. Examples of conservative variations include the substitution of one hydrophobic residue such as isoleucine, valine, leucine or methionine for another hydrophobic residue, or the substitution of one polar residue for another polar residue, such as the substitution of arginine for lysine, glutamic acid for aspartic acid, or glutamine for asparagine, and the like; or a similar conservative replacement of an amino acid with a structurally related amino acid that will not have a major effect on the biological activity. Proteins having substantially the same amino acid sequence as the reference molecule but possessing minor amino acid substitutions that do not substantially affect the immunogenicity of the protein are, therefore, within the definition of the reference polypeptide. All of the polypeptides produced by these modifications are included herein. The term "conservative variation" also includes the use of a substituted amino acid in place of an unsubstituted parent amino acid provided that antibodies raised to the substituted polypeptide also immunoreact with the unsubstituted polypeptide.

The term "epitope" refers to the site on an antigen or hapten to which specific B cells and/or T cells respond. The term is also used interchangeably with "antigenic determinant" or "antigenic determinant site". Antibodies that recognize the

same epitope can be identified in a simple immunoassay showing the ability of one antibody to block the binding of another antibody to a target antigen.

An "immunological response" to a composition or vaccine is the development in the host of a cellular and/or antibody-mediated immune response to a composition or vaccine of interest. Usually, an "immunological response" includes but is not limited to one or more of the following effects: the production of antibodies, B cells, helper T cells, and/or cytotoxic T cells, directed specifically to an antigen or antigens included in the composition or vaccine of interest. Preferably, the host will display either a therapeutic or protective immunological response such that resistance to new infection will be enhanced and/or the clinical severity of the disease reduced. Such protection will be demonstrated by either a reduction or lack of symptoms normally displayed by an infected host, a quicker recovery time and/or a lowered viral titer in the infected host.

The terms "recombinant" and "genetically modified" are used interchangeably and refer to any modification, alteration or engineering of a polynucleotide or protein in its native form or structure, or any modification, alteration or engineering of a polynucleotide or protein in its native environment or surrounding. The modification, alteration or engineering of a polynucleotide or protein may include, but is not limited to, deletion of one or more nucleotides or amino acids, deletion of an entire gene, codon-optimization of a gene, conservative substitution of amino acids, insertion of one or more heterologous polynucleotides.

The term "double HVT construct" or "double HVT vector" refers to an HVT viral vector comprising two heterologous polynucleotides.

The terms "polyvalent vaccine or composition", "combination or combo vaccine or composition" and "multivalent vaccine or composition" are used interchangeably to refer to a composition or vaccine containing more than one composition or vaccines. The polyvalent vaccine or composition may contain two, three, four or more compositions or vaccines. The polyvalent vaccine or composition may comprise recombinant viral vectors, active or attenuated or killed wild-type viruses, or a mixture of recombinant viral vectors and wild-type viruses in active or attenuated or killed forms.

One embodiment of the invention provides a recombinant HVT viral vector comprising one or more heterologous polynucleotides coding for and expressing at least one antigen or polypeptide of an avian pathogen. The HVT strains used for the recombinant viral vector may be any HVT strains, including, but not limited to, the HVT strain FC126 (Igarashi T. et al., J. Gen. Virol. 70, 1789-1804, 1989).

Another embodiment of the invention provides a recombinant SB-1 viral vector comprising one or more heterologous polynucleotides coding for and expressing at least one antigen or polypeptide of an avian pathogen. The SB-1 strains may be any SB-1 strains, including, but not limited to, the commercial Marek's Disease Vaccine (SB-1 vaccine) (Merial Select Inc., Gainesville, Ga. 30503, USA), the SB-1 strain having the genome sequence as defined by GenBank Accession Number HQ840738.1.

The genes coding for antigen or polypeptide may be those coding for Newcastle Disease Virus fusion protein (NDV-F), Newcastle Disease Virus hemagglutinin neuraminidase (NDV-HN), Marek's Disease Virus glycoprotein C (gC), Marek's Disease Virus glycoprotein B (gB), Marek's Disease Virus glycoprotein E (gE), Marek's Disease Virus glycoprotein I (gI), Marek's Disease Virus glycoprotein H (gH) or Marek's Disease Virus glycoprotein L (gL), Infectious Bursal Disease Virus (IBDV) VP2, IBDV VPX, IBDV VP3, IBDV

VP4, ILTV glycoprotein B, ILTV glycoprotein I, ILTV UL32, ILTV glycoprotein D, ILTV glycoprotein E, ILTV glycoprotein C, influenza hemagglutinin (HA), influenza neuramidase (NA), protective genes derived from *Mycoplasma gallisepticum* (MG), or *Mycoplasma synoviae* (MS), or combinations thereof. The antigen or polypeptide may be any antigen from the poultry pathogen selected from the group consisting of avian encephalomyelitis virus, avian reovirus, avian paramyxovirus, avian metapneumovirus, avian influenza virus, avian adenovirus, fowl pox virus, avian coronavirus, avian rotavirus, chick anemia virus, avian astrovirus, avian parvovirus, coccidiosis (*Eimeria* sp.), *Campylobacter* sp., *Salmonella* sp., *Pasteurella* sp., *Avibacterium* sp., *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, *Clostridium* sp., and *E. coli*.

Moreover, homologs of aforementioned antigen or polynucleotides are intended to be within the scope of the present invention. As used herein, the term "homologs" includes orthologs, analogs and paralogs. The term "analogs" refers to two polynucleotides or polypeptides that have the same or similar function, but that have evolved separately in unrelated organisms. The term "orthologs" refers to two polynucleotides or polypeptides from different species, but that have evolved from a common ancestral gene by speciation. Normally, orthologs encode polypeptides having the same or similar functions. The term "paralogs" refers to two polynucleotides or polypeptides that are related by duplication within a genome. Paralogs usually have different functions, but these functions may be related. Analogs, orthologs, and paralogs of a wild-type polypeptide can differ from the wild-type polypeptide by post-translational modifications, by amino acid sequence differences, or by both. In particular, homologs of the invention will generally exhibit at least 80-85%, 85-90%, 90-95%, or 95%, 96%, 97%, 98%, 99% sequence identity, with all or part of the polynucleotide or polypeptide sequences of antigens described above, and will exhibit a similar function.

In one embodiment, the present invention provides a recombinant HVT or SB-1 viral vector comprising one or more heterologous polynucleotides coding for and expressing the NDV-F antigen or polypeptide. In one aspect of the embodiment, the NDV-F antigen or polypeptide has at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity to a polypeptide having the sequence as set forth in SEQ ID NO:2, 4, 6, 33, 35, or 37, or a conservative variant, an allelic variant, a homolog or an immunogenic fragment comprising at least eight or at least ten consecutive amino acids of one of these polypeptides, or a combination of these polypeptides. In another aspect of the embodiment, the heterologous polynucleotide encoding an NDV-F antigen or polypeptide having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity to a polypeptide having the sequence as set forth in SEQ ID NO:2, 4, 6, 33, 35, or 37. In yet another aspect of the embodiment, the heterologous polynucleotide has at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity to a polynucleotide having the sequence as set forth in SEQ ID NO:1, 3, 5, 32, 34, or 36.

Variants include allelic variants. The term "allelic variant" refers to a polynucleotide or a polypeptide containing polymorphisms that lead to changes in the amino acid sequences of a protein and that exist within a natural population (e.g., a virus species or variety). Such natural allelic variations can typically result in 1-5% variance in a polynucleotide or a polypeptide. Allelic variants can be identified by sequencing the nucleic acid sequence of interest in a number of different species, which can be readily carried out by using hybridiza-

tion probes to identify the same gene genetic locus in those species. Any and all such nucleic acid variations and resulting amino acid polymorphisms or variations that are the result of natural allelic variation and that do not alter the functional activity of gene of interest, are intended to be within the scope of the invention.

The term "identity" with respect to sequences can refer to, for example, the number of positions with identical nucleotides or amino acids divided by the number of nucleotides or amino acids in the shorter of the two sequences wherein alignment of the two sequences can be determined in accordance with the Wilbur and Lipman algorithm (Wilbur and Lipman). The sequence identity or sequence similarity of two amino acid sequences, or the sequence identity between two nucleotide sequences can be determined using Vector NTI software package (Invitrogen, 1600 Faraday Ave., Carlsbad, Calif.). When RNA sequences are said to be similar, or have a degree of sequence identity or homology with DNA sequences, thymidine (T) in the DNA sequence is considered equal to uracil (U) in the RNA sequence. Thus, RNA sequences are within the scope of the invention and can be derived from DNA sequences, by thymidine (T) in the DNA sequence being considered equal to uracil (U) in RNA sequences.

The polynucleotides of the disclosure include sequences that are degenerate as a result of the genetic code, e.g., optimized codon usage for a specific host. As used herein, "optimized" refers to a polynucleotide that is genetically engineered to increase its expression in a given species. To provide optimized polynucleotides coding for NDV-F polypeptides, the DNA sequence of the NDV-F protein gene can be modified to 1) comprise codons preferred by highly expressed genes in a particular species; 2) comprise an A+T or G+C content in nucleotide base composition to that substantially found in said species; 3) form an initiation sequence of said species; or 4) eliminate sequences that cause destabilization, inappropriate polyadenylation, degradation and termination of RNA, or that form secondary structure hairpins or RNA splice sites. Increased expression of NDV F protein in said species can be achieved by utilizing the distribution frequency of codon usage in eukaryotes and prokaryotes, or in a particular species. The term "frequency of preferred codon usage" refers to the preference exhibited by a specific host cell in usage of nucleotide codons to specify a given amino acid. There are 20 natural amino acids, most of which are specified by more than one codon. Therefore, all degenerate nucleotide sequences are included in the disclosure as long as the amino acid sequence of the NDV-F polypeptide encoded by the nucleotide sequence is functionally unchanged.

Successful expression of the heterologous polynucleotides by the recombinant/modified infectious virus requires two conditions. First, the heterologous polynucleotides must be inserted or introduced into a region of the genome of the virus in order that the modified virus remains viable. The second condition for expression of inserted heterologous polynucleotides is the presence of a regulatory sequences allowing expression of the gene in the viral background (for instance: promoter, enhancer, donor and acceptor splicing sites and intron, Kozak translation initiation consensus sequence, polyadenylation signals, untranslated sequence elements).

The insertion site may be any non-essential region of the HVT genome, including, but not limited to, the region between the ATG of ORF UL55 and the junction of UL with the adjacent repeat region (U.S. Pat. No. 5,980,906), the IG1 locus, the IG2 locus, the IG3 locus, the UL43 locus, the US10 locus, the SORF3/US2 locus (see FIG. 2)

In general, it is advantageous to employ a strong promoter functional in eukaryotic cells. The promoters include, but are not limited to, an immediate early cytomegalovirus (CMV) promoter, guinea pig CMV promoter, an SV40 promoter, Pseudorabies Virus promoters such as that of glycoprotein X promoter, Herpes Simplex Virus-1 such as the alpha 4 promoter, Marek's Disease Viruses (including MDV-1, MDV-2 and HVT) promoters such as those driving glycoproteins gC, gB, gE, or gI expression, Infectious Laryngotracheitis Virus promoters such as those of glycoprotein gB, gE, gI, gD genes, or other herpesvirus promoters.

One embodiment of the invention provides a recombinant HVT vector comprising a heterologous polynucleotide coding for and expressing the NDV-F antigen or polypeptide. In one aspect of the embodiment, the polynucleotide encoding the NDV-F polypeptide is operably linked to the SV40 promoter having the sequence as set forth in SEQ ID NO:9 and therefore the expression of the NDV-F antigen or polypeptide is regulated by the SV40 promoter. In another aspect of the embodiment, the expression of NDV-F antigen or polypeptide is regulated by the SV40 polyA signal having the sequence as set forth in SEQ ID NO:11. In yet another aspect of the embodiment, the polynucleotide encoding the NDV-F polypeptide is operably linked to the MDV gB promoter having the sequence as set forth in SEQ ID NO:38 and therefore the expression of the NDV-F antigen or polypeptide is regulated by the MDV gB promoter.

Another embodiment of the invention provides a recombinant double HVT vector comprising a first heterologous polynucleotide coding for and expressing the NDV-F antigen or polypeptide and a second polynucleotide coding for and expressing the IBDV VP2 antigen or polypeptide. In one aspect of the embodiment, the NDV-F antigen or polypeptide has at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity to a polypeptide having the sequence as set forth in SEQ ID NO:2, 4, 6, 33, 35, or 37. In another aspect of the embodiment, the IBDV VP2 antigen or polypeptide has at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity to a polypeptide having the sequence as set forth in SEQ ID NO:8 or 42. In another aspect, the polynucleotide encoding the NDV-F polypeptide is operably linked to the SV40 promoter having the sequence as set forth in SEQ ID NO:9 and the expression of NDV-F antigen or polypeptide is regulated by the SV40 promoter. In yet another aspect, the expression of NDV-F antigen or polypeptide is regulated by the SV40 polyA signal having the sequence as set forth in SEQ ID NO:11, or the synthetic polyA signal having the sequence as set forth in SEQ ID NO:12. In another aspect, the expression of IBDV VP2 antigen or polypeptide is regulated by the CMV-IE promoter having the sequence as set forth in SEQ ID NO:10 and the SV40 polyA signal having the sequence as set forth in SEQ ID NO:11.

Yet another embodiment of the invention provides a recombinant double HVT vector comprising two polynucleotides coding for and expressing the IBDV VP2 antigens or polypeptides. In one aspect of the embodiment, the IBDV VP2 antigen or polypeptide has at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity to a polypeptide having the sequence as set forth in SEQ ID NO:8 or 42. In one aspect, the polynucleotide encoding a first IBDV VP2 antigen or polypeptide is operably linked to the CMV-IE promoter having the sequence as set forth in SEQ ID NO:10, and the polynucleotide encoding a second IBDV VP2 antigen or polypeptide is operably linked to the guinea pig CMV promoter having the sequence as set forth in SEQ ID NO:43. In another aspect, the expression of a first IBDV VP2 antigen

or polypeptide is regulated by the CMV-IE promoter having the sequence as set forth in SEQ ID NO:10 and the SV40 polyA signal having the sequence as set forth in SEQ ID NO:11, and the expression of a second IBDV VP2 antigen or polypeptide is regulated by the guinea pig CMV promoter having the sequence as set forth in SEQ ID NO:43 and the synthetic polyA signal having the sequence as set forth in SEQ ID NO:12. In yet another aspect of the embodiment, the polynucleotides encoding the IBDV VP2 antigen or polypeptide may be inserted in one or more locus regions selected from the group consisting of IG1, IG2, US10, SORF3-US2 and gD of HVT genome. In one embodiment, the present invention relates to a pharmaceutical composition or vaccine comprising one or more recombinant HVT or SB-1 raval vectors of the present invention and a pharmaceutically or veterinarianily acceptable carrier, excipient, vehicle or adjuvant.

In another embodiment, the present invention provides a composition or vaccine comprising an HVT viral vector comprising a polynucleotide encoding an NDV-F antigen, an SV40 promoter, and optionally a pharmaceutically or veterinarianily acceptable carrier, excipient, vehicle or adjuvant. In another embodiment, the present invention provides a pharmaceutical composition or vaccine comprising a first HVT vector comprising a polynucleotide encoding an NDV-F antigen, a second HVT vector comprising a polynucleotide encoding an IBDV VP2 antigen, and optionally a pharmaceutically or veterinarianily acceptable carrier, excipient, vehicle or adjuvant. In another embodiment, the present invention provides a pharmaceutical composition or vaccine comprising an HVT vector comprising a polynucleotide encoding an NDV-F antigen, an SB-1 vector comprising a polynucleotide encoding an NDV-F antigen, optionally a pharmaceutically or veterinarianily acceptable carrier, excipient, vehicle or adjuvant. The pharmaceutical composition or vaccine of the present invention may comprise a first HVT vector comprising a polynucleotide encoding an NDV-F antigen, a second HVT vector comprising a polynucleotide encoding an IBDV VP2 antigen, an SB-1 vector comprising a polynucleotide encoding an NDV-F antigen, optionally a pharmaceutically or veterinarianily acceptable carrier, excipient, vehicle or adjuvant.

In yet another embodiment, the present invention provides a composition or vaccine comprising a double HVT viral vector comprising: i) a first heterologous polynucleotide coding for and expressing an NDV-F antigen or polypeptide; ii) a second polynucleotide coding for and expressing an IBDV VP2 antigen or polypeptide; and iii) optionally a pharmaceutically or veterinarianily acceptable carrier, excipient, vehicle or adjuvant. In another embodiment, the present invention provides a composition or vaccine comprising a double HVT viral vector comprising two polynucleotides coding for and expressing the IBDV VP2 antigens or polypeptides, and optionally a pharmaceutically or veterinarianily acceptable carrier, excipient, vehicle or adjuvant. In yet another embodiment, the composition comprising the double HVT viral vector further comprises an HVT vector comprising a polynucleotide encoding an IBDV VP2 antigen, or an SB-1 vector comprising a polynucleotide encoding an NDV-F antigen, or a combination thereof. The pharmaceutically or veterinarianily acceptable carriers or adjuvant or vehicles or excipients are well known to the one skilled in the art. For example, a pharmaceutically or veterinarianily acceptable carrier or adjuvant or vehicle or excipient can be Marek's disease vaccine diluent used for MD vaccines. Other pharmaceutically or veterinarianily acceptable carrier or adjuvant or vehicle or excipients that can be used for methods of this invention include, but are not limited to, 0.9% NaCl (e.g., saline) solution or a phosphate buffer, poly-(L-glutamate) or polyvi-

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nylpyrrolidone. The pharmaceutically or veterinarily acceptable carrier or vehicle or excipients may be any compound or combination of compounds facilitating the administration of the vector (or protein expressed from an inventive vector *in vitro*), or facilitating transfection or infection and/or improve preservation of the vector (or protein). Doses and dose volumes are herein discussed in the general description and can also be determined by the skilled artisan from this disclosure read in conjunction with the knowledge in the art, without any undue experimentation.

Optionally other compounds may be added as pharmaceutically or veterinarily acceptable carriers or adjuvant or vehicles or excipients, including, but not limited to, alum; CpG oligonucleotides (ODN), in particular ODN 2006, 2007, 2059, or 2135 (Pontarollo R. A. et al., *Vet. Immunol. Immunopath.*, 2002, 84: 43-59; Wernette C. M. et al., *Vet. Immunol. Immunopath.*, 2002, 84: 223-236; Mutwiri G. et al., *Vet. Immunol. Immunopath.*, 2003, 91: 89-103); polyA-polyU, dimethyldioctadecylammonium bromide (DDA) ("Vaccine Design The Subunit and Adjuvant Approach", edited by Michael F. Powell and Mark J. Newman, *Pharmaceutical Biotechnology*, 6: p. 03, p. 157); N,N-dioctadecyl-N',N'-bis(2-hydroxyethyl) propanediamine (such as AVRIDINE®) (*Ibid.*, p. 148); carbomer, chitosan (see U.S. Pat. No. 5,980,912 for example).

The pharmaceutical compositions and vaccines according to the invention may comprise or consist essentially of one or more adjuvants. Suitable adjuvants for use in the practice of the present invention are (1) polymers of acrylic or methacrylic acid, maleic anhydride and alkenyl derivative polymers, (2) immunostimulating sequences (ISS), such as oligodeoxyribonucleotide sequences having one or more non-methylated CpG units (Klinman et al., 1996; WO98/16247), (3) an oil in water emulsion, such as the SPT emulsion described on p 147 of "Vaccine Design, The Subunit and Adjuvant Approach" published by M. Powell, M. Newman, Plenum Press 1995, and the emulsion MF59 described on p 183 of the same work, (4) cation lipids containing a quaternary ammonium salt, e.g., DDA (5) cytokines, (6) aluminum hydroxide or aluminum phosphate, (7) saponin or (8) other adjuvants discussed in any document cited and incorporated by reference into the instant application, or (9) any combinations or mixtures thereof.

Another aspect of the invention relates to a method for inducing an immunological response in an animal against one or more antigens or a protective response in an animal against one or more avian pathogens, which method comprises inoculating the animal at least once with the vaccine or pharmaceutical composition of the present invention. Yet another aspect of the invention relates to a method for inducing an immunological response in an animal to one or more antigens or a protective response in an animal against one or more avian pathogens in a prime-boost administration regimen, which is comprised of at least one primary administration and at least one booster administration using at least one common polypeptide, antigen, epitope or immunogen. The immunological composition or vaccine used in primary administration may be same, may be different in nature from those used as a booster.

The avian pathogens may be Newcastle Disease Virus (NDV), Infectious Bursal Disease Virus (i.e., IBDV or Gumboro Disease virus), Marek's Disease Virus (MDV), Infectious Laryngotracheitis Virus (ILTV), avian encephalomyelitis virus, avian reovirus, avian paramyxovirus, avian metapneumovirus, avian influenza virus, avian adenovirus, fowl pox virus, avian coronavirus, avian rotavirus, avian parvovirus, avian astrovirus and chick anemia virus coccidioides

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(*Eimeria* sp.), *Campylobacter* sp., *Salmonella* sp., *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, *Pasteurella* sp., *Avibacterium* sp., *E. coli* or *Clostridium* sp.

Usually, one administration of the vaccine is performed either at one day-of-age by the subcutaneous or intramuscular route or *in ovo* in 17-19 day-old embryo. A second administration can be done within the first 10 days of age. The animals are preferably at least 17 day-embryo or one day old at the time of the first administration.

A variety of administration routes in day-old chicks may be used such as subcutaneously or intramuscularly, intradermally, transdermally. The *in ovo* vaccination can be performed in the amniotic sac and/or the embryo. Commercially available *in ovo* and SC administration devices can be used for vaccination.

The invention will now be further described by way of the following non-limiting examples.

EXAMPLES

Construction of DNA inserts, plasmids and recombinant viral vectors was carried out using the standard molecular biology techniques described by J. Sambrook et al. (*Molecular Cloning: A Laboratory Manual*, 2nd Edition, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1989).

Example 1

Construction of Recombinant vHVT114 Expressing NDV-F

Preparation of Donor Plasmid pHM103+Fopt

The plasmid pHM103 (Merial Limited) containing the Intergenic I arms of HVT FC126 (see FIG. 2), SV40 promoter and SV40 poly A was digested with NotI, dephosphorylated, and the 5.6 kb fragment was gel extracted. A NotI flanked 1.7 kb fragment of a chemically synthesized codon-optimized genotype VIIId NDV-F gene (SEQ ID NO:1, coding for SEQ ID NO:2) was also NotI digested and the 1.7 kb fragment was gel extracted. The 5.6 and 1.7 kb fragments were ligated to create pHM103+Fopt (FIG. 3).

Generation of Recombinant HVT Viral Vector

An *in vitro* recombination (IVR) was performed by co-electroporation of secondary chicken embryo fibroblast cells (2° CEF cells) using pHM103+Fopt as the donor plasmid and viral DNA isolated from the HVT strain FC126. Co-electroporation was performed using 1×10⁷ 2° CEF in 300 ul Opti-MEM and shocked at 150 volts with 950 capacitance in a 2 mm electroporation cuvette. The transfected cells were seeded into 96-well plate and incubated for 5 days. The cells grown in the 96-well plate were then duplicated into two 96-well plates. One set of 96-well plates was used for IFA using chicken polyclonal sera against NDV-F to identify positive wells containing recombinants and another set of 96-well plates was used for recovering the infected cells from the positive wells.

The recombinant viral purification was performed first by 96-well plate duplication and IFA selection for the wells containing the most IFA positive plaques with the least amount of IFA negative plaques. Wells matching those criteria were then harvested and adjusted to 1 ml in DMEM+2% FBS. From the 1 ml stock, 5-20 ul were removed and mixed with 1×10⁷ CEFs in 10 ml DMEM+2% FBS and aliquoted onto a new 96-well plate to have single HVT plaques per well. The supernatant of the wells that contained single plaques were tested for the absence of parental virus by PCR. After five rounds of plaque purification, a recombinant virus des-

ignated as vHVT114 was isolated and the purity was tested by IFA and PCR to confirm NDV-F expression and the absence of parental virus.

PCR Analysis of Recombinant vHVT114

DNA was extracted from vHVT114 by phenol/chloroform extraction, ethanol precipitated, and was resuspended in 20 mM HEPES. PCR primers (shown in Table 1) were designed to specifically identify the presence of the codon optimized NDV-F, the SV40 promoter, as well as, the purity of the recombinant virus from FC126 CL2 parental virus. PCR was performed using 200 ng of DNA template along with the specified primers pairs indicated in Table 1. PCR cycling conditions are as follows: 94° C. for 2 mins; 30 cycles of 94° C. for 30 secs, 55° C. for 30 secs, 68° C. for 3 mins; 68° C. for 5 mins. The expected PCR products are shown in Table 2. The PCR results are shown in FIG. 4. As shown in FIG. 4, the sizes of PCR products after gel electrophoresis correspond well with the expected sizes and the banding patterns.

TABLE 1

primer	SEQ ID NO	Sequence 5'-3'
MB080	13	CGA ACA AAC TTC ATC GCT ATG C
MB081	14	TAA CTC AAA TGC GAA GCG TTG C
optF	15	ACT GAC AAC ACC CTA CAT GGC
VIIoptF RP	16	GCC AGC ACC AGG CTC AGG G
SV40promoterF	17	AGC TTG GCT GTG GAA TGT

TABLE 2

Primer pairs	Expected size (bp)	
	FC126 CL21	vHVT114
MB081 + VIIoptF.RP	—	2138
SV40promoterF + MB080	—	2368
OptF primer + MB080	—	872
MB080 + MB081	323	2578

Expression Analysis of Recombinant vHVT114

Immunofluorescence testing was performed using the vHVT114 which was passaged over ten times beyond an experimental pre-master seed (pre-MSV). The pre-MSV and pre-MSV+12 materials were diluted 1:100 in media. Fifty microliters of the diluted virus was added to 10 ml of DMEM+2% FBS with 1×10^7 CEFs and then aliquoted onto a 96 well plate (100 μ l/well). The plates were incubated for 3 days at 37° C.+5% CO₂ until viral plaques were visible. The plates were fixed with 95% ice-cold acetone for three minutes and washed three times with PBS. Chicken anti-sera against Newcastle Disease Virus (lot#C0139, Charles Rivers Laboratory) at 1:1000 were added along with monoclonal antibody L-78 (Merial Limited) at 1:3000 and the plates were incubated at 37° C. for 1 hour. After the 1 hour incubation the plates were washed three times with PBS and FITC anti-chicken (cat# F8888, Sigma) was added along with Alexz Fluor 568 donkey anti-mouse (IgG) (cat# A 10037, Molecular Probe) at 1:500. Again the plates were incubated at 37° C. for 1 hour. After the 1 hour incubation the cells were rinsed three times with PBS. A small amount of PBS was added to prevent the monolayer from drying and causing auto fluorescence. The cells were then visualized with a fluorescent microscope

using both the tetramethylrhodamine isothiocyanate (TRITC) and fluorescein isothiocyanate (FITC) filters in combination.

The vHVT114 viral plaques were visualized using both the TRITC and FITC filters for the dual staining. The FITC test showed the NDV-F expression and the TRITC test showed the HVT expression. Because of the small wells of the 96 well plates, each well was recorded with the plaques first counted with the TRITC filter and then recounted with the FITC filter. Over 500 plaques were counted for the pre-MSV and pre-MSV+12 passage. All the plaques were positive for both the FITC and TRITC on both plates. (FIG. 5)

Southern Blot Analysis of Recombinant vHVT114

Total genomic DNA was extracted from HVT FC126 and vHVT114 according to the standard genomic DNA extraction protocol. For each restriction digest, 3 μ g of genomic DNA (1 ng for the donor plasmid) was used with a total digestion volume of 20 μ l for each sample. The genomic DNA of HVT FC126 (negative control), pHM103+Fopt donor plasmid, and vHVT114 were each digested overnight at 37° C. with BamHI, PstI, SphI, and NcoI restriction endonucleases. The restriction fragments of HVT FC126 (negative control), pHM103+Fopt donor plasmid, and vHVT114 genomic DNA were separated by a 1% agarose gel and transferred to a positively charged Nylon membrane. Following the North2South Chemiluminescent Hybridization and Detection Kit (Thermo Scientific) manufacturers' instructions, the membrane was pre-hybridized for 1 hr and then hybridized with a biotinylated NDV-F probe overnight at 55° C. Following the overnight hybridization, several stringency washes were performed until the membrane was placed in blocking buffer with the addition of Streptavidin-HRP. After rinsing the membrane of any unbound Streptavidin-HRP the substrate solution of Luminal and peroxide were added. The membrane was then exposed to X-ray film and developed. Areas where the biotinylated probe bound to the DNA were chemiluminescent and were captured by the X-ray film. Table 3 shows the expected Southern blot bands using the NDV-F probe. The Southern blot results showed the digestion patterns as expected (FIG. 6).

TABLE 3

Restriction Endonuclease	NDV-F Probe		
	Donor plasmid pHM103 + Fopt	vHVT114	FC126 CL2
BamHI	7.014	6.630	—
	0.198	1.259	—
	—	0.198	—
PstI	5.481	6.359	—
	0.947	0.947	—
	0.784	0.784	—
SphI	4.763	2.377	—
	2.377	2.119	—
	0.072	0.072	—
NcoI	4.931	3.753	—
	2.157	2.157	—
	0.124	0.124	—

Sequence Analysis of the Inserted Region in Recombinant vHVT114

Analysis of vHVT114 genomic DNA region was performed by PCR amplification. Total of 10 primers were used to amplify the entire cassette, as well as, beyond the flanking BamHI-I arms used in the donor plasmid. The 4.727 kb PCR product was gel purified and the entire fragment was sequenced using the sequencing primers. The sequence result confirmed that the vHVT114 contains the correct SV40 pro-

moter, the codon-optimized NDV-F and the SV40 polyA sequences that match exactly the sequence described for the donor plasmid pHM103+Fopt in SEQ ID NO:18.

Western Blot Analysis of Recombinant vHVT114

Approximately 2×10^6 chicken fibroblast cells were infected at ~0.1 MOI with vHVT114 Pre-MSV. After two days of incubation at 37°C, infected as well as uninfected cells were harvested using a cell scraper after removing the media and rinsing with PBS. The cells were harvested with 1 ml of PBS and centrifuged. The cell pellets were lysed by following the Pierce Classic IP Kit (cat#26146, Thermo Scientific). 100 µl of the anti-NDV-F monoclonal antibody 001C3 (Merial Limited) was used to form the immune complex. The antibody/lysate sample was added to Protein A/G Plus Agarose to capture the immune complex. The immune complex was washed three times to remove non-bound material and then eluted in 50 µl volume using sample buffer elution under non-reducing condition. After boiling for 5 minutes, 10 µl of the samples were loaded into a 10% Acrylamide gel (Invitrogen). The PAGE gel was run in MOPS buffer (Invitrogen) at 200 volts for 1 hour. Then the gel was transferred onto a PVDF membrane.

The Protein Detector Western Blot Kit TMB System (KPL, cat#54-11-50) was used for blotting the PVDF membrane by using the reagents and following manufacturer's directions. After blocking the membrane for 1 hour at room temperature, the membrane was then rinsed three times in 1× Wash Buffer, five minutes each and then soaked in blocking buffer containing 1:1000 dilution of chicken serum raised against NDV virus (Lot # C0139, Charles River Laboratories). After washing three times in a washing buffer, the membrane was incubated with a peroxidase labeled goat anti-chicken IgG (KPL, cat#14-24-06) at a dilution of 1:2000 for 1 hour at room temperature. The membrane was then rinsed three times in 1× Wash Buffer, five minutes each. 5 ml of TMB membrane peroxidase substrate was added to the membrane and gently rocked for about 1 minute. The developing reaction was stopped by placing the membrane into water.

The immunoprecipitation and Western blot technique detected an approximately 55 kD protein in vHVT114 sample that corresponds to the expected size of F1 component of the NDV-F protein (FIG. 7).

Example 2

Construction of Recombinant vHVT110, vHVT111, vHVT112, vHVT113 and vHVT116 Expressing NDV-F

Generation and characterization of HVT recombinants vHVT110, vHVT111, vHVT112, vHVT113, and vHVT116 was essentially done in the same way as for vHVT114 described in example 1. Table 4 shows the features unique to each construct around the expression cassettes, including the respective sequences.

TABLE 4

Characteristics of the expression cassettes of single HVT recombinants					
Name	Parental virus	Promoter	F gene	Poly-A	Locus
vHVT039	HVT	MDV gB	Wtnm-Texas	SV40	IG1
vHVT110	HVT	mCMV IE	Wt-VIIId	SV40	IG1
vHVT111	HVT	SV40	Wt-VIIId	SV40	IG1
vHVT112	HVT	MCMV IE	Wt-YZCQ	SV40	IG1

TABLE 4-continued

Characteristics of the expression cassettes of single HVT recombinants						
5	Name	Parental virus	Promoter	F gene	Poly-A	Locus
vHVT113	HVT	MCMV IE	Wt-Texas	SV40	IG1	
vHVT114	HVT	SV40	Opt-VIIId	SV40	IG1	
vHVT116	HVT	SV40	Opt-Ca02	SV40	IG1	

vHVT110

The plasmid pCD046 (Merial proprietary material) containing the Intergenic I arms of HVT FC126, mouse CMV promoter and SV40 poly A was digested with NotI, dephosphorylated, and a 6.6 kb fragment was gel extracted. A NotI flanked 1.7 kb fragment of a chemically synthesized NDV-F gene containing wild-type F sequence (SEQ ID NO:3, coding for SEQ ID NO:4) was also NotI digested and the 1.7 kb fragment was gel extracted. The 6.6 and 1.7 kb fragments were ligated to create a donor plasmid pCD046+NDV-F wt (SEQ ID NO:21 for vHVT110) used in transfection to generate recombinant vHVT110. Sequencing of the insert region confirmed that vHVT110 contains the correct sequences of mCMV promoter, the wildtype NDV-F gene and the SV40 polyA. The sequence also exactly matches the sequence described for the donor plasmid pCD046+NDV-F wt in SEQ ID NO:21.

vHVT111

The plasmid pHM103 plasmid (Merial proprietary material) containing the Intergenic I arms of HVT FC126, SV40 promoter and SV40 polyA was digested with NotI, dephosphorylated, and the 5.6 kb fragment was gel extracted. A NotI flanked 1.7 kb fragment of a chemically synthesized NDV-F gene containing wildtype F sequence (SEQ ID NO:3, coding for SEQ ID NO:4) was also NotI digested and a 1.7 kb fragment was gel extracted. The 5.6 and 1.7 kb fragments were ligated to create a donor plasmid (SEQ ID NO:22 for vHVT111) used in transfection to generate recombinant vHVT111. Sequencing of the insert region confirmed that vHVT111 contains the correct sequences of SV40 promoter, the wildtype NDV-F gene and the SV40 polyA as shown in the sequence of the donor plasmid pHM103+NDV-F wt (SEQ ID NO:22).

vHVT112

A fragment encompassing the synthetic NDV-F YZCQ wild type gene (SEQ ID NO:34 encoding SEQ ID NO:35) was excised from pUC57 NDV-F YZCQ plasmid (synthesized by GeneScript) using NotI and inserted into the same site of pCD046 plasmid containing mCMV promoter and SV40 polyA tail. Ligated material was transformed using Top10 Oneshot kit (cat#C404002, Invitrogen). Bacterial colonies were grown in LBamp broth, plasmid extracted by using Qiagens MiniSpin Prep kit, and screened for insert orientation. The correct donor plasmid was designated pCD046+NDV-F VII YZCQ. Large scale cultures were grown and plasmid extraction was done by using Qiagens Maxi Prep kit. Transient expression of the maxi preps was verified using Fugene Transfection Reagent in Chicken Embryo Fibroblast Cells (CEF's) and chicken polyclonal sera against NDV.

Plasmid pCD046+NDV-F VII YZCQ (SEQ ID NO:29) was used in transfection to generate recombinant vHVT112. Sequencing of the insert region confirmed that vHVT112 contains the correct sequences of mCMV promoter, the wild-type NDV-F YZCQ gene and the SV40 polyA. The sequence

also exactly matches the sequence described for the donor plasmid pCD046+NDV-F VII YZCQ in SEQ ID NO:29. vHVT113

A fragment encompassing the synthetic NDV Texas F gene (SEQ ID NO:36 encoding SEQ ID NO:37) was excised from pUC57 NDV Texas F plasmid (synthesized by GeneScript) using NotI and inserted into the same site of pCD046 plasmid containing mCMV promoter and SV40 polyA tail. Ligated material was transformed using Top10 Oneshot kit (cat#C404002, Invitrogen). Bacterial colonies were grown in LBamp broth, plasmid extracted by using Qiagens MiniSpin Prep kit, and screened for insert orientation. The correct donor plasmid was designated pCD046+Texas NDV-F. Large scale cultures were grown and plasmid extraction was done by using Qiagens Maxi Prep kit. Transient expression of the maxi preps was verified using Fugene Transfection Reagent in Chicken Embryo Fibroblast Cells (CEF's) and chicken polyclonal sera against NDV.

Plasmid pCD046+Texas NDV-F (SEQ ID NO:30) was used in transfection to generate recombinant vHVT113. Sequencing of the insert region confirmed that vHVT113 contains the correct sequences of mCMV promoter, the wild-type NDV-F Texas F gene and the SV40 polyA. The sequence also exactly matches the sequence described for the donor plasmid pCD046+Texas NDV-F in SEQ ID NO:30. vHVT039

The MDV gB promoter (SEQ ID NO:38) was amplified from MDV1 RB1B strain extracted DNA by PCR using the primers HM101 (5'-CCG-GAA-TTC-CGA-TGT-TTA-GTC-ACG-ATA-GAC-3') (SEQ ID NO:44) and HM102 (5'-ATA-AGA-GCG-GCC-GCA-GTG-AGA-TGA-TCT-TAA-TGA-TG-3') (SEQ ID NO:45). The former contains an EcoRI site and the latter contains a NotI site for ligation of the EcoRI/NotI digested 630 bp PCR product into EcoRI/NotI digested pCD046 plasmid. The ligation product was used to transform DH5 α competent cells. Colonies were picked and screened for the presence of the inserted PCR fragment by restriction analysis with EcoRI and NotI. The resulting plasmid was designated pHM102.

The velogenic NDV Texas strain (genotype IV) was grown on 11-day-old SPF embryonated eggs and semi-purified. Total RNA was extracted and an RT PCR was performed using two primers F-ATG (5' TAT-AGC-GGC-CGC-AAG-ATG-GGC-TCC-AGA-TCT-TCT-ACC-AG 3') (SEQ ID NO:46) and F-STOP (5' CGA-GGC-GGC-CGC-TCA-TAT-TTT-TGT-AGT-GGC-TCT-C 3') (SEQ ID NO:47). They allow the whole amplification of the NDV F gene with addition of NotI site upstream ATG and downstream STOP codons. The 1.7 kb PCR fragment was digested with NotI and ligated into NotI-digested pHM102. The resulting plasmid was designated pHM119 and was used as a donor plasmid in in vitro recombination study by co-transfection of CEF cells with HVT parental DNA to generate vHVT039 as described above. Sequencing of the insert region confirmed that vHVT039 contains the correct sequences of MDV gB promoter, the wildtype unmodified NDV-F gene from Texas strain (SEQ ID NO:32 encoding SEQ ID NO:33) and the SV40 polyA as shown in the partial sequence of the donor plasmid pHM119 (SEQ ID NO:31). vHVT116

The plasmid pHM103 plasmid (Merial proprietary material) containing the Intergenic I arms of HVT FC126, SV40 promoter and SV40 polyA was digested with NotI, dephosphorylated, and the 5.6 kb fragment was gel extracted. A NotI flanked 1.7 kb fragment of a chemically synthesized, codon-optimized, CA02 genotype V NDV-F gene (SEQ ID NO:5, coding for SEQ ID NO:6) was also NotI digested and the 1.7

kb fragment was gel extracted. The 5.6 and 1.7 kb fragments were ligated to create pHM103+NDV-F CA02 (SEQ ID NO:23 for vHVT116) used in transfection to generate recombinant vHVT116. Sequencing of the insert region confirmed that vHVT116 contains the correct sequences of SV40 promoter, the codon-optimized CA02 NDV-F gene and the SV40 polyA as shown in the sequence of the donor plasmid pHM103+NDV-F wt (SEQ ID NO:23).

Discussion

Various cassettes under mCMV or non-CMV promoter were inserted at different loci of HVT genome (Table 4). Despite repeated attempts, generating a construct with a combination of mCMV and codon-optimized F sequence was not successful beyond passage 2. However, when wild-type sequence was driven by mCMV a stable construct, vHVT110 could be generated. In addition, recombinant vHVT111 with wild-type F sequence under SV40 promoter was also stable for more than 10 in vitro passages. Surprisingly, a codon-optimized F sequence under SV40 promoter was similarly found to be stable for more than 10 in vitro passages (e.g. vHVT114 and vHVT116). These results indicate the delicate balance between the strength of the promoter and the nature of the gene they control (codon-optimized or not optimized) in generating a genetically stable HVT construct.

Example 3

Construction of vHVT306, a Double HVT Vector Expressing NDV-F and IBDV VP2

The donor plasmid pHVT US2 SV-Fopt-synPA was constructed containing SV40 promoter, synthetic NDV F codon optimized VII gene, synthetic polyA tail flanked by the SORF3 and US2 arm sequences of HVT FC126.

Generation of Recombinant Virus

A standard homologous recombination procedure was followed by co-electroporation of secondary CEF cells using donor plasmid pHVT US2 SV-Fopt-synPA and viral DNA isolated from vHVT13 (an HVT vector expressing the IBDV VP2 gene, Merial Limited). Essentially the procedure described in example 1 for vHVT114 was followed to generate, plaque purify and characterize recombinants by immunofluorescence.

After five rounds of plaque purification, pure recombinant virus (vHVT306) was isolated and the purity of vHVT306 was tested and confirmed by IFA and PCR.

PCR Analysis

Viral DNA was extracted from vHVT306 pre-master seed virus (pre-MSV) stock by QIA DNeasy Blood & Tissue Kit (Qiagen cat#69506). PCR primers were designed to identify the presence of the NDV F optimized, the NDV F wild type, the SV40 promoter, the mCMV promoter, the flanking arms of US2 HVT virus and SB-1 virus.

PCR amplification with various primers confirmed that the vHVT306 has the expected amplification patterns and amplicons.

Expression Analysis

Indirect immunofluorescent assay (IFA) was performed on the vHVT306 pre-MSV stock. The CEFs that were inoculated with vHVT306 were fixed with ice-cold 95% acetone for three minutes at room temperature and air-dried for 10 min. After three washes with PBS, two primary antibodies, chicken anti-Newcastle Disease Virus sera (Charles Rivers Laboratories cat#10100641, lot#C0117A) at 1:500 dilution and L78 monoclonal antibody against HVT (Merial Select, Gainesville, Ga.) at 1:3000 dilution were added and incubated for 45 min at 37 °C. After three washes with PBS, two secondary antibodies, goat anti-chicken IgG-fluorescein (KPL cat#02-24-06, lot#110020) at 1:500 dilution and donkey anti-mouse IgG-Alexa Fluor 568 (Molecular Probe

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#A10037, lot#989784) at 1:300 dilution were added. The plates were incubated at 37° C. for 45 min and followed by three washes with PBS. The cells were observed to identify the IFA positive plaques with a fluorescent microscope using fluorescein isothiocyanate (FITC)- and tetramethylrhodamine isothiocyanate (TRITC)-filters of Nikon Eclipse Ti inverted microscope.

Similarly the expression of IBDV VP2 protein (SEQ ID NO:8 encoded by SEQ ID NO:7) of vHVT306 were examined by IFA using chicken anti-IBDV sera (Charles River Laboratories cat#10100610 lot#G0117) (1:500 dilution) and anti-NDV F monoclonal antibody 001C3 (Asceitic fluid, Batch 10/09/044, 02/11/2010) (1:300 dilution) as primary antibodies; followed by goat anti-chicken IgG-fluorescein (KPL cat#.02-24-06, lot#110020) (1:500 dilution) and donkey anti-mouse IgG-Alexa Fluor 568 (Molecular Probe #A10037, lot#989784) (1:300 dilution) as secondary antibodies.

IFA results indicate that vHVT306 expresses the NDV F genes in virus-infected CEFs.

Over 400 vHVT306 plaques were counted using the FITC-filter and TRITC-filter of microscope. The overall expression of NDV F gene and IBDV VP2 match with the HVT plaques (Table 5).

TABLE 5

Dual IFA of vHVT306				
Virus	IFA #1 (total 453 plaques)		IFA#2 (total 478 plaques)	
	Anti-NDV serum positive plaques	Anti-HVT MAb positive plaques	Anti-NDV F MAb positive plaques	Anti-IBDV serum positive plaques
vHVT306 pre-MSV	453	453	478	478

Southern Blot Analysis

Total genomic DNA was extracted from vHVT306 pre-MSV stock infected CEFs. The Southern blot analysis was performed according to the standard protocol.

A total 3 probes were used to confirm the NDV F cassette (SV40 promoter, NDV F codon optimized gene, synthetic polyA tail) between SORF3 and US2 of vHVT306 as well as retention of IBDV VP2 cassette (mCMV promoter, IBDV VP2 gene, SV40 poly A tail).

The Southern blot results showed the digestion patterns as expected based on Vector NTI (Invitrogen, 1600 Faraday Ave., Carlsbad, Calif.) map analysis. The NDV F cassette (SV40 promoter, NDV F codon optimized gene, synthetic poly A tail) is located between SORF3 and US2, and IBDV VP2 cassette (mCMV promoter, IBDV VP2 gene, SV40 poly A tail) is intact like the parent virus (vHVT13).

Genomic Analysis

The genomic DNA of vHVT306 pre-MSV stock was sequenced to verify the sequence of the recombination arm region as well as inserted gene cassette.

Primers were designed to amplify the entire inserted gene cassette including recombination arm used in donor plasmid. Analysis of vHVT306 genomic DNA was performed by PCR amplification and followed by nucleotide sequence determination.

The vHVT306 (donor plasmid pHVT US2 SV-Fopt-synPA) containing the recombinant arms, SV40 promoter and NDV F codon-optimized gene was confirmed to be correct as shown in SEQ ID NO:20.

Western Blot Analysis

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The CEF monolayer was infected with vHVT306 pre-MSV at MOI ~0.1. After a 4-day incubation, the CEFs were pelleted and washed with PBS followed by lysis with IP Lysis/Wash buffer of Pierce Classic IP Kit (Thermo Scientific cat#26146) according to the manufacturer's protocols. The lysate was pre-cleared and incubated with 100 ul of anti-NDV F monoclonal antibody 001C3 to make the immune complex. The immune complex was captured by Protein A/G Plus Agarose and after removing of the un-bounded immune complex by washing steps, the 50 ul of sample buffer was used to elute under non-reducing conditions. The uninfected CEFs were included as controls. The 20 ul of eluted samples were separated in a 10% Bis-Tris Gels by electrophoresis. After the electrophoresis, the separated proteins were transferred onto PVDF membrane. The Protein Detection TMB Western Blot Kit (KPL cat#54-11-50) was used to detect the NDV antigens on PVDF membrane with chicken anti-NDV serum (Charles River Laboratories cat#10100641, lot#C0117A), and goat anti-chicken IgG-peroxidase conjugate (KPL. cat#414-24-06) following the manufacturers' protocols.

The NDV F protein expression of vHVT306 was confirmed by two-step immunodetection. First, the expressed NDV F proteins from vHVT306 infected CEF were captured by the immunoprecipitation using anti-NDV F monoclonal antibody 001C3. Subsequently Western blot analysis using anti-NDV polyclonal serum (Charles River Laboratories cat#10100641, lot#C0117A) was applied to detect the NDV F protein in the captured samples (NDV F protein-monoclonal antibody complex) (FIG. 8). A 55 kDa protein in vHVT306 pre-MSV lysates was detected by anti-NDV serum which corresponds to the expected size of NDV F1 fusion protein (FIG. 8).

Example 4

Construction of Double HVT Vectors vHVT301, vHVT302, vHVT303, vHVT304 and vHVT307 Expressing NDV-F and IBDV VP2, and Double HVT Vector vHVT202 Expressing IBDV VP2 Variants

Example 4.1

Construction of vHVT301, vHVT302, vHVT303, vHVT304 and vHVT307

Generation and characterization of double HVT recombinants vHVT301, vHVT302, vHVT303, vHVT304, and vHVT307 were essentially done in the same way as for vHVT306 described in example 3. Table 6.1 shows the features unique to each construct around the expression cassettes, including the respective sequences.

TABLE 6.1

Characteristics of the expression cassettes of double HVT recombinants					
Name	Parental virus	Promoter	NDV-F gene	Poly-A	Locus
vHVT301	vHVT13	SV40	Wt-VIIId NDV-F	SV40	IG2
vHVT302	vHVT13	US10	Opt-VIIId NDV-F	US10	US10
vHVT303	vHVT13	US10	Opt-V NDV-F	US10	US10
vHVT304	vHVT13	SV40	Opt-VIIId NDV-F	Synthetic	IG2

TABLE 6.1-continued

Characteristics of the expression cassettes of double HVT recombinants					
Name	Parental virus	Promoter	NDV-F gene	Poly-A	Locus
vHVT306	vHVT13	SV40	Opt-VIId NDV-F	Synthetic	SORF3-US2
vHVT307	vHVT13	SV40	Opt-V NDV-F	Synthetic	SORF3-US2

vHVT301

The plasmid pHVT IG2 SbfI (Merial proprietary material) containing the Intergenic 2 arm sequences of vHVT13 was digested with SmaI, dephosphorylated, and the 4.3 kb fragment was gel extracted. The donor plasmid pHM103+NDV-F wt containing an SV40 promoter, wildtype NDV-F genotype VIId, SV40 poly A tail was EcoRI and SalI digested, klenow treated, and the 2.3 kb fragment was gel extracted. The two fragments were ligated to create a donor plasmid pHVT IG2 SV Fwt SbfI (SEQ ID NO: 24) used in transfection to generate recombinant vHVT301.

vHVT302

A synthetically synthesized plasmid, pHVT US10 cds, containing the US10 arm sequences of vHVT13 was digested with NotI, dephosphorylated, and the 4.7 kb fragment was gel extracted. A NotI flanked 1.7 kb fragment of a chemically synthesized, codon-optimized, NDV-F genotype VIId was NotI digested and gel extracted. The two fragments were ligated to create a donor plasmid pHVT US10 cds F opt used in transfection to generate recombinant vHVT302. The transcription of the inserted F gene should be driven by the native US10 promoter and be stopped by the native US10 polyA signal. No exogenous promoter or polyA is added to express this insert. Sequencing of the insert region confirmed that vHVT302 contains the correct sequence of the codon-optimized VIId NDV-F gene as shown in the sequence of the donor plasmid pHVT US10 cds F opt (SEQ ID NO: 25).

vHVT303

The synthetically synthesized plasmid pHVT US10 cds containing the US10 arm sequences of vHVT13 was digested with NotI, dephosphorylated, and the 4.7 kb fragment was gel extracted. A NotI flanked 1.7 kb fragment of a chemically synthesized, codon-optimized, NDV-F genotype V was NotI digested and gel extracted. The two fragments were ligated to create a donor plasmid pHVT US10 cds F CAO2 opt used in transfection to generate recombinant vHVT303. As with vHVT302, the transcription of this inserted F gene should also be driven by the native US10 promoter and be stopped by the native US10 polyA signal. No exogenous promoter or polyA is added to express this insert. Sequencing of the insert region confirmed that vHVT303 contains the correct sequence of the codon-optimized NDV-F genotype V as shown in the sequence of the donor plasmid pHVT US10 cds F CAO2 (SEQ ID NO: 26).

vHVT304

The donor plasmid pHVT IG2 SbfI containing the Intergenic 2 arm sequences of vHVT13 was digested with SbfI, dephosphorylated, and the 4.3 kb fragment was gel extracted. A synthetically synthesized plasmid containing an SV40 promoter+codon optimized NDV-F genotype VIId+synthetic polyA tail flanked by SbfI was digested with SbfI and the 2.3 kb fragment was gel extracted. The two fragments were ligated to create a donor plasmid pHVT IG2 SV Fopt syn tail used in transfection to generate recombinant vHVT304. Sequencing of the insert region confirmed that vHVT304

contains the correct sequences of SV40 promoter, the codon-optimized VIId NDV-F gene, and the synthetic poly A tail as shown in the sequence of the donor plasmid pHVT IG2 SV Fopt syn tail (SEQ ID NO:27).

5 vHVT307

The donor plasmid pHVT US2-SORF3 containing the US2 and SORF3 arm sequences of vHVT13 was digested with SbfI, dephosphorylated, and the 5.1 kb fragment was gel extracted. The plasmid SB-1 UL55 SV CaF syn tail SbfI 10 containing an SV40 promoter+codon optimized NDV-F genotype V+synthetic polyA tail flanked by SbfI was digested with SbfI and the 2.3 kb fragment was gel extracted. The two fragments were ligated to create a donor plasmid pHVT US2 SV-FCA02 opt-synPA used in transfection to 15 generate recombinant vHVT307. Sequencing of the insert region confirmed that vHVT307 contains the correct sequences of SV40 promoter, the codon-optimized VIId NDV-F gene, and the synthetic poly A tail as shown in the sequence of the donor plasmid pHVT US2 SV-FCA02 opt-synPA (SEQ ID NO: 28).

Discussion

One of the main goals of this work was to develop a multivalent avian Herpesvirus-based vector by incorporating multiple protective genes of interest to one avian Herpesvirus backbone (e.g. HVT). A prerequisite for this approach is to define expression cassettes containing appropriate promoter-gene-polyA combinations and evaluate for their genetic stability and ability to protect against the specific disease.

For the purpose of creating an efficacious MD-IBD-ND 30 trivalent vector vaccine, either codon-optimized or non-optimized Newcastle Disease Virus (NDV)-F gene sequences were cloned into vHVT13 backbone (HVT-IBD, a licensed vaccine to simultaneously protect chickens against MD and IBD) under human CMV (mouse CMV is already used in 35 vHVT13). All vHVT-IBD-F constructs under human CMV promoter lost F-protein expression within six passages whether or not the NDV-F sequence is codon-optimized and regardless of the insertion site. The loss of F protein expression was rapid (within two passes) when hCMV was combined with codon-optimized F protein as compared to a combination of hCMV with wild-type F-sequence (loss of F 40 protein expression within 6 passages). Taken together, the data shows that human CMV is not an ideal promoter for the generation of stable HVT recombinants expressing NDV-F protein. Surprisingly, this example shows that SV40 promoter and HVT endogenous promoter (US10 promoter) generated 45 stable HVT recombinants expressing NDV-F protein.

Example 4.2

Construction of vHVT202

Donor Plasmid HVT SORF3-US2 gpVar-Ewtsyn Construction

55 A fragment encompassing the synthetic Varient E wild type IBDV VP2 gene (SEQ ID NO:41 encoding SEQ ID NO:42) was excised from pUC57 Varient E wt plasmid (synthesized by GeneScript) using NotI and inserted into the same site of SORF3 and US2 plasmid containing gpCMV promoter and 60 synthetic polyA tail. Ligated material was transformed using Top10 Oneshot kit (cat#C404002, Invitrogen). Bacterial colonies were grown in LBamp broth, plasmid extracted by using Qiagens MiniSpin Prep kit, and screened for insert orientation using SacI+HindIII digestion. The correct donor 65 plasmid was designated pHVT SORF3-US2 gpVar-Ewt Syn. Table 6.2 shows the features unique to the construct around the expression cassettes, including the respective sequences.

Large scale cultures were grown and plasmid extraction was done by using Qiagens Maxi Prep kit. Transient expression of the maxi preps was verified using Fugene Transfection Reagent in Chicken Embryo Fibroblast Cells (CEF's) and chicken polyclonal sera against IBDV.

TABLE 6.2

Characteristics of the expression cassettes of double HVT recombinants				
Name	Parental virus	Promoter	IBDV VP2 gene	Poly-A Locus
vHVT202	vHVT306	Guinea pig CMV	IBDV E VP2	Synthetic SORF3-US2

Recombinant Generation

A standard homologous recombination procedure was followed by co-electroporation of secondary CEF cells using pHVTSORF3-US2 gpVar-Ewt Syn donor plasmid and viral DNA isolated from vHVT306 and digested with SbfI. vHVT306, expressing classical VP2 of IBDV and NDV-F, was chosen as a parent to simplify the section process as described below. The variant E VP2 donor plasmid was designed to replace the F gene and recombinants were initially selected for the absence of F gene expression and later by PCR for the presence of variant EVP2. Co-electroporation was performed using 1×10^7 2° CEF in 300 μ l Opti-MEM and shocked at 150 volts with 950 capacitance in a 2 mm electroporation cuvette. The transfected cells were seeded into 96-well plate and incubated for 5-7 days. The cells grown in the 96-well plate were then duplicated into two 96-well plates and incubated for 5 more days. One set of 96-well plates was used for IFA using chicken polyclonal sera against NDV-F to identify positive wells containing the vHVT306 parents and another set of 96-well plates was used for recovering the infected cells from the IFA negative wells.

The recombinant viral purification methods were performed first by 96-well plate duplication and IFA selection for the wells containing the most IFA negative (against NDV-F) plaques with the least amount of IFA positive plaques. Wells matching those criteria were then harvested and adjusted to 1 ml in DMEM+2% FBS. From the 1 ml stock 5-20 μ l (depending on the number of visible plaques) were removed and mixed with 1×10^7 CEFs in 10 ml DMEM+2% FBS and aliquoted onto a new 96-well plate in an attempt to have single HVT plaques per well. The 96-well plates were duplicated after 4 days of incubation and wells that contained plaques were tested for the presence of recombinant HVT and absence of parental virus by IFA and PCR. Again the wells that appeared to have more recombinant virus and less parent virus, by comparing the PCR banding results, were harvested and adjusted to 1 ml and aliquoted onto new 96-well plates (the same as before). After five rounds of purification of virus infected cells, recombinant HVT carrying two IBDV VP2 proteins was isolated and the purity of the recombinant virus was tested by PCR to confirm the absence of parental virus.

Sequencing of the insert region confirmed that vHVT202 contains the correct sequences of guinea pig CMV promoter, the IBDV Varient E wildtype VP2 gene, and the synthetic poly A tail as shown in the sequence of the donor plasmid HVT SORF3-US2 gpVar-Ewtsyn (SEQ ID NO:39).

Analysis of Recombinant by PCR

DNA was extracted from a stock virus by phenol/chloroform extraction, ethanol precipitated, and resuspended in 20 mM HEPES. PCR primers were designed to specifically iden-

tify the Varient E wt gene, the promoter, the polyA, as well as, the purity of the recombinant virus from HVT parental virus. PCR was performed using 200 μ g of DNA template along with the specified primers pairs indicated in Table 1. PCR cycling conditions are as follows (unless otherwise noted): 94° C.-2 min; 30 cycles of 94° C.-30 sec, 55° C.-30 sec, 68° C.-3 min; 68° C.-5 min.

Purity of recombinant virus was verified by PCR using primer pairs that are specific to the HVT flanking arms, the gpCMV promoter, the Varient E gene and the syn tail. Primers, specific to SB1, MDV serotype 2 (SB1US1.FP+SB1Sorf4.RP) were also included in the analysis. The PCR results demonstrate that recombinant virus vHVT202 carries the intended expression cassette and the virus stock is free from detectable amounts of parental HVT virus.

Immunofluorescent Staining of Recombinant vHVT202 Virus Expressing Two VP2 Proteins of IBDV

For immunofluorescence testing, the P3 material was diluted 1:100 in media. Approximately 50 μ l of the diluted virus was added to 10 ml of DMEM+2% FBS with 1×10^7 CEFs and then aliquoted onto a 96 well plate (100 μ l/well). The plates were incubated for 4 days at 37° C.+5% CO₂ until viral plaques were visible. The plates were fixed with 95% ice-cold acetone for three minutes and washed three times with PBS. One well was used for chicken anti-sera against Newcastle Disease Virus (lot#C0139, Charles Rivers Laboratory) at 1:1000 was added and the plates were incubated at 37° C. for 1 hour. The other well was used for chicken anti-sera against IBDV (lot#G0117) After one hour incubation, the plates were washed three times with PBS and FITC anti-chicken (cat# F8888, Sigma) was added at 1:500. Again the plates were incubated at 37° C. for 1 hour. After one hour incubation the cells were rinsed three times with PBS and visualized with a fluorescent microscope using fluorescein isothiocyanate (FITC) filter.

The immunofluorescent staining results indicate that vHVT202 exhibited a very strong expression of the VP2 protein when the polyclonal sera against both classical and variant E VP2 proteins were used.

Conclusion

Based on PCR and immunofluorescence analysis, vHVT202 is a recombinant HVT in which a VP2 gene of variant E IBDV under the control of gpCMV promoter was successfully inserted into a recombinant HVT background that already expresses the VP2 gene of classical IBDV. Consequently vHVT202 carries both VP2 genes of variant E and classical IBDV and it is free of any detectable parental vHVT306 virus.

Example 5

Construction of Recombinant vSB1-009, vSB1-004, vSB1-006, vSB1-007, vSB1-008, and vSB1-010 Expressing NDV-F

Example 5.1

Construction of vSB1-009, vSB1-004, vSB1-006, vSB1-007, and vSB1-008

The aim of the study is to construct a recombinant SB-1 viral vector vSB1-009 in which an expression cassette containing SV40 promoter and Newcastle disease virus fusion protein (NDV-F) is inserted to replace UL44 coding sequence (gC) of SB-1.

25

A donor plasmid pSB1 44 cds SV FCAopt was constructed containing UL44 flanking arms of SB1 virus, SV40 promoter and NDV F codon optimized gene sequence (SEQ ID NO:5, coding for SEQ ID NO:6).

Generation of Recombinant Virus

A standard homologous recombination procedure was followed by co-electroporation of secondary CEF cells using donor plasmid pSB1 44 cds SV FCAopt and viral DNA isolated from SB-1 virus infected CEFs. Essentially the procedure described in example 1 for vHVT114 was followed to generate, plaque purify and characterize recombinants by immunofluorescence.

After five rounds of plaque purification, pure recombinant virus (vSB1-009) was isolated and the purity of vSB1-009 was tested by IFA and PCR to validate the appropriate insertion as well as no remnant parental virus.

PCR Analysis

Viral DNA was extracted from vSB1-009 pre-master seed virus (pre-MSV) stock by QIA DNeasy Blood & Tissue Kit (Qiagen cat#69506). PCR primers were designed to identify the presence of the NDV F optimized, the NDV F wild type, the SV40 promoter, the mCMV promoter, the UL44 flanking arms of SB-1 virus and HVT virus. PCR amplifications were performed using approximately 200 ng of DNA template along with the primer pairs.

PCR amplification with various primers confirmed that the vSB1-009 has the expected amplification patterns and amplicons.

Expression Analysis

Indirect immunofluorescent assay (IFA) was performed on the vSB1-009 pre-MSV stock to examine the expression of NDV F gene and SB-1 virus antigen. The CEFs that were inoculated with vSB1-009 were fixed with ice-cold 95% acetone for three minutes at room temperature and air-dried for 10 min. The plates were washed with PBS, then two primary antibodies, chicken anti-Newcastle Disease Virus sera (Charles Rivers Laboratories cat#10100641, lot#C0117A) at 1:500 dilution and Y5.9 monoclonal antibody against SB-1 virus (Merial Select, Gainesville, Ga.) at 1:3000 dilution were added and the plates were incubated for 45 min at 37° C. After three washes with PBS, two secondary antibodies, goat anti-chicken IgG-fluorescein (KPL cat#.02-24-06, lot#110020) at 1:500 dilution and donkey anti-mouse IgG-Alexa Fluor 568 (Molecular Probe #A10037, lot#989784) at 1:250 dilution were added. The plates were incubated at 37° C. for 45 min and followed by three washes with PBS. The wells were screened for IFA positive plaques with a fluorescent microscope using fluorescein isothiocyanate (FITC) and tetramethylrhodamine isothiocyanate (TRITC)-filters of Nikon Eclipse Ti inverted microscope. Similarly, reactivity of vSB1-009 with NDV F Mab was examined by Dual IFA using anti-MDV serum (Charles River Laboratories, cat#10100628, lot#D0111) (1/300 dilution) and anti-NDV F monoclonal antibody (1/300 dilution) as primary antibody. The goat anti-chicken IgG-fluorescein (KPL cat#.02-24-06, lot#110020) (1:500 dilution) and donkey anti-mouse IgG-Alexa Fluor 568 (Molecular Probe #A10037, lot#989784) (1:250 dilution) were used as secondary antibodies. The wells were observed to identify the IFA positive plaques with a fluorescent microscope using FITC and TRITC-filters of Nikon Eclipse Ti inverted microscope.

IFA results indicate that vSB1-009 expresses the NDV F protein in virus-infected CEF. Over 500 vSB1-009 plaques were counted for NDV F protein expression as well as SB-1 virus specific protein expression with dual IFA. The expression of NDV F protein completely matched with SB-1 virus antigen expression in each virus plaque (Table 7).

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TABLE 7

Virus	Dual IFA of vSB1-009			
	Dual IFA plate#1 (total 189 plaques)		Dual IFA plate#2 (total 361 plaques)	
	Anti-NDV serum positive plaques	Anti-SB-1 MAb positive plaques	Anti-NDV serum positive plaques	Anti-SB-1 MAb positive plaques
vSB1-009 pre-MSV	189	189	361	361

NDV F Mab reactivity was confirmed by Dual IFA. Over 200 vSB1-009 plaques were examined for NDV F Mab reactivity as well as anti-MDV serum reactivity. The reactivity with NDV F Mab completely matched with anti-MDV serum reactivity in each virus plaque (Table 8).

TABLE 8

Virus	Reactivity of vSB1-009 with anti-NDV F Mab	
	Dual IFA (total 254 plaques)	
	Anti-MDV serum positive plaques	Anti-NDV F MAb positive plaques
vSB1-009 pre-MSV	254	254

Southern Blot Analysis

Total genomic DNA was extracted from vSB1-009 pre-MSV stock infected CEFs. The genomic DNA of vSB1-009, SB-1 virus (negative control), pSB1 44 cds SV FCA opt donor plasmid were digested at 37° C. with EcoRI, NcoI, and KpnI restriction endonucleases separately. The restriction fragments were separated by a 0.8% agarose gel electrophoresis and transferred onto a positively charged Nylon membrane. After transfer, the membrane was treated with 0.4M NaOH and then neutralized with 2×SSC-HCl buffer. The membrane was then air dried and UV crosslinked.

Following the North2South Chemiluminescent Hybridization and Detection Kit (Thermo Scientific cat#89880) manufacturers' instructions, the membrane was pre-hybridized for 1 hr and then hybridized with the probe at 55° C. for overnight. For hybridization, two probes were used; 1) the SbfI fragment of pSB1 44 cds SV FCA opt as NDV F cassette probe, 2) the SmaI-EcoRI fragment of pUC57 SB1 44 arm (GenScript) as recombination arm probe. After the overnight hybridization, several stringency washes were conducted until the membrane was placed in blocking buffer with the addition of Streptavidin-HRP. After rinsing the membrane of any unbound Streptavidin-HRP, the substrate solution of Luminal and peroxide were added. The membrane was then exposed to X-ray film and the film was developed.

The Southern blot results were as expected based on Vector NTI map analysis. The NDV F cassette (SV40 promoter, NDV-F CA02 codon optimized gene) replaced the UL44 coding sequences of SB-1 virus.

Genomic Analysis

The genomic DNA of vSB1-009 pre-MSV stock was conducted by nucleotide sequence determination of the region of recombination arm as well as inserted gene cassette. Primers were designed and used to amplify the entire NDV-F gene cassette including the recombination arms.

The vSB1-009 sequence (donor plasmid pSB1 44 cds SV FCAopt) containing the recombinant arms, SV40 promoter and NDV F codon-optimized gene was confirmed to be correct as shown in SEQ ID NO:19.

Western Blot Analysis

The CEF monolayer was infected with vSB1-009 pre-MSV at MOI ~0.1. After a 5-day incubation, the CEFs were pelleted and washed with PBS followed by lysis with IP Lysis/Wash buffer of Pierce Classic IP Kit (Thermo Scientific cat#26146) according to the manufacturers' protocols. The lysate was pre-cleared and incubated with 100 µl of anti-NDV F monoclonal antibody to make the immune complex. The immune complex was captured by Protein A/G Plus Agarose and after removing of the un-bounded immune complex by washing steps, the 50 µl of sample buffer was used to elute under non-reducing conditions. The uninfected CEFs were included as a control. The 20 µl of eluted samples were separated in 10% Bis-Tris gels by electrophoresis. After the electrophoresis, the separated proteins in a gel were transferred onto PVDF membrane. The Protein Detection TMB Western Blot Kit (KPL cat#54-11-50) was used to detect the NDV antigens onto PVDF membrane with chicken anti-NDV serum (Charles River Laboratories cat#10100641, lot#C0117A), and goat anti-chicken IgG-peroxidase conjugate (KPL cat#14-24-06) following the manufacturers' protocols.

The NDV F protein expression of vSB1-009 was confirmed by two-step immunodetection. First, the expressed NDV F proteins from vSB1-009 infected CEF lysate were captured by the immunoprecipitation using anti-NDV F monoclonal antibody 001C3. Subsequently Western blot analysis using anti-NDV polyclonal serum (Charles River Laboratories cat#10100641, lot#C0117A) was applied to detect the NDV F protein in the captured samples (NDV F protein-monoconal antibody complex) (FIG. 9). An approximately 55 kDa protein in vSB1-007 pre-MSV lysates was detected by anti-NDV serum that corresponding the expected size of NDV F1 fusion protein (FIG. 9).

Generation and characterization of HVT recombinants vSB1-004, vSB1-006, vSB1-007 and vSB1-008 were essentially done in the same way as for vSB1-009 described in this example. Table 9.1 shows the features unique to each construct around the expression cassettes, including the respective sequences. The generation and characterization of recombinant SB1 viral vectors were also described in U.S. patent application Ser. No. 13/689,572 filed on Nov. 29, 2012 (Merial limited), which is incorporated herein by reference in its entirety.

TABLE 9.1

Characteristics of the expression cassettes of SB1 recombinants				
Name	Parental virus	Promoter	F gene	Locus
vSB1-009	SB1	SV40	Opt-CA02	UL44 (gC)
vSB1-004	SB1	mCMV IE	Wt-VIIId	US10
vSB1-006	SB1	SV40	Opt-VIIId	UL55/LORF5
vSB1-007	SB1	SV40	Opt-VIIId	UL44 (gC)
vSB1-008	SB1	SV40	Opt-CA02	UL55/LORF5

Example 5.2

Construction of Double Construct vSB1-010

Donor Plasmid SB1US2 gpVIIIdwtsyn Construction

Using the plasmid HVT SOrf3-US2 gpVar-Ewt Syn, the gpCMV, Varient E, Syn tail was removed by SbfI digestion. This fragment was ligated into the SB1 US2 donor plasmid. The Varient E gene was cut out by NotI and replaced by

NDV-F VIIId wt. The synthetic NDV-F VIIId wild type gene (SEQ ID NO:3 encoding SEQ ID NO:4) was excised from pUC57 NDV-F VIIId wt plasmid (synthesized by GeneScript) using NotI digestion. Ligated material was transformed using Top10 Oneshot kit (cat#C404002, Invitrogen). Bacterial colonies were grown in LBamp broth, plasmid extracted by using Qiagens MiniSpin Prep kit, and screened for insert orientation using NcoI+Sall digestion. The correct donor plasmid was designated pSB1 US2 gpVIIIdwtsyn. Table 9.2 shows the features unique to the construct around the expression cassettes, including the respective sequences. Large scale cultures were grown and plasmid extraction was done by using Qiagens Maxi Prep kit. Transient expression of the maxi preps was verified using Fugene Transfection Reagent in Chicken Embryo Fibroblast Cells (CEF's) and chicken polyclonal sera against NDV-F.

Recombinant Generation

A standard homologous recombination procedure was followed by co-electroporation of secondary CEF cells using pSB1 US2 gpVIIIdwtsyn donor plasmid and viral DNA isolated from vSB1-009 (vSB1-009 is already a recombinant virus expressing CA02 F gene of NDV). Essentially the procedure described in example 1 for vHVT114 was followed to generate, plaque purify and characterize recombinants by immunofluorescence.

After five rounds of plaque purification, pure recombinant virus (vSB1-010) was isolated and the purity of vSB1-010 was tested by IFA and PCR to validate the appropriate insertion as well as no remnant parental virus.

TABLE 9.2

Characteristics of the expression cassette of vSB1-010				
35 Name	Parental virus	Promoter	F gene	Locus
vSB1-010	vSB1-009	Guinea pig CMV	NDV-F VIIId	SORF4-US2

40 Sequencing of the insert region confirmed that vSB1-010 contains the correct sequences of guinea pig CMV promoter and the NDV-F VIIId wt gene as shown in the sequence of the donor plasmid SB1US2 gpVIIIdwtsyn (SEQ ID NO:40). Analysis of Recombinant by PCR

45 DNA was extracted from a stock virus by phenol/chloroform extraction, ethanol precipitated, and resuspended in 20 mM HEPES. PCR primers were designed to specifically identify the NDV-F VIIId wt gene, the promoter, the polyA, as well as, the purity of the recombinant virus from SB1 parental virus. PCR was performed using 200 µg of DNA template along with the specified primers pairs indicated in Table 1. PCR cycling conditions are as follows (unless otherwise noted): 94° C.-2 min; 30 cycles of 94° C.-30 sec, 55° C.-30 sec, 68° C.-3 min; 68° C.-5 min.

55 Purity of recombinant virus was verified by PCR using primer pairs that are specific to the SB1 flanking arms, the gpCMV promoter, the NDV-F VIIId wt gene and the syn tail. Primers, specific to HVT, MDV serotype 3 (MB080+MB081) were also included in the analysis. The PCR results demonstrate that recombinant virus vSB1-010 carries the intended expression cassette and the virus stock is free from detectable amounts of parental SB1-009 virus.

Immunofluorescent Staining of Recombinant vSB1-010 Virus Expressing Two NDV-F Proteins

60 For immunofluorescence testing, the P3 material was diluted 1:100 in media. Approximately 50 µl of the diluted virus was added to 10 ml of DMEM+2% FBS with 1×10⁷

CEFs and then aliquoted onto a 96 well plate (100 µl/well). The plates were incubated for 5 days at 37° C.+5% CO₂ until viral plaques were visible. The plates were fixed with 95% ice-cold acetone for three minutes and washed three times with PBS. Chicken anti-sera against Newcastle Disease Virus (lot#C0139, Charles Rivers Laboratory) at 1:1000 was added and the plates were incubated at 37° C. for 1 hour. After one hour incubation, the plates were washed three times with PBS and FITC anti-chicken (cat# F8888, Sigma) was added at 1:500. Again the plates were incubated at 37° C. for 1 hour. After one hour incubation the cells were rinsed three times with PBS and visualized with a fluorescent microscope using fluorescein isothiocyanate (FITC) filter.

The immunofluorescent staining results indicate that vSB1-010 exhibited a very strong expression of the NDV-F protein when the polyclonal sera against both CA02 and VIIId F proteins of NDV were used.

Conclusion

Based on PCR testing and immunofluorescence analysis, vSB1-010 is a recombinant SB-1 in which VIIId-F gene of NDV under the control of gpCMV promoter was successfully inserted into a vSB1-009, which already expresses the CA02-F gene of NDV. Consequently vSB1-010 carries both VIIId and CA02 F genes of NDV genotypes and it is free of any detectable parental vSB1-009.

Example 6

Efficacy of vHVT110, vHVT111, vHVT114 and vSB1-004 Expressing the NDV F Gene Against Challenges with NDV Chimalhuacan and Malaysian (MAL04-01) Strains at 14 Days of Age in SPF Chickens

The aim of the study was to assess the efficacy of three HVT recombinant constructs (vHVT110, vHVT111 and vHVT114) and one SB1 recombinant construct (vSB1-004) expressing the NDV F gene against Newcastle disease challenges (Chimalhuacan and Malaysian virus strains) performed at 14 days of age in SPF chickens.

The characteristics of these 5 vaccine candidates are described in Table 10 below.

TABLE 10

Characteristics of the vectors used in the challenge study					
Name	Parental virus	Promoter	F gene	Poly-A	Locus
vHVT110	HVT	mCMV IE	Wt-VIIId	SV40	IG1
vHVT111	HVT	SV40	Wt-VIIId	SV40	IG1
vHVT114	HVT	SV40	Opt-VIIId	SV40	IG1
vSB1-004	SB-1	mCMV IE	Wt-VIIId	SV40	US10

On D0, 100 one-day-old SPF chickens were randomly allocated into 10 groups of 10 birds. The birds were injected by subcutaneous injection in the neck at D0 with 0.2 mL of recombinant vaccines containing a target dose of 2000 pfu as described in Table 11 below. It should be mentioned that the titer of vSB1-004 (31600 pfu) administered to birds of groups 6 was well above the target. The birds were challenged by the intramuscular route on D14 with velogenic ND Malaysia (genotype VIIId) strain (sub-groups "a") or with virulent ND Chimalhuacan (genotype V) strain (sub-groups "b").

TABLE 11

5	Challenge study with vHVT110, vHVT111, vHVT114 and vSB1-004				
	Group	Vaccine at day-old	NDV serology	% protection against mortality/morbidity after Newcastle challenge at 14 days of age (D14)	
		(D0)	at D14*	Malaysian strain	Chimalhuacan strain
10	G1a	—	0/10	0%/0%	—
	G1b	—	—	—	0%/0%
	G2a	vHVT110	7/10	100%/89%	—
	G2b	vHVT110	—	—	100%/70%
	G3a	vHVT111	2/10	30%/20%	—
	G3b	vHVT111	—	—	67%/11%
	G4a	vHVT114	9/10	100%/100%	—
	G4b	vHVT114	—	—	89%/89%
	G5a	vSB1-004	3/10	70%/50%	—
	G5b	vSB1-004	—	—	40%/30%

*Number of birds positive by NDV HI test/total tested

Each group was monitored before and after challenge. Clinical signs after challenge were scored daily as follows: healthy/with specific symptoms (ruffled feathers, prostration, torticollis, tremor)/dead. On D14, serum samples were taken in each group for serology (Newcastle Disease virus hemagglutination inhibition (HI) test).

As expected, the unvaccinated animals (G1a and G1b) displayed no NDV antibodies on D14. A low titer seroconversion (mean HI titer <0.6 log 10) was obtained in each vaccinated group (sub-groups "a" and "b" of G2 to G5) confirming the vaccine takes. The number of positive birds/total tested was group-dependent and was the highest (90%) in vHVT114 vaccinated birds (see Table above).

Percentages of protection against mortality and morbidity are reported in the table above. Full susceptibility was observed in the control groups G1a and G1b thus validating the high severity of both challenges. Lowest protection levels were observed in the groups vaccinated with vHVT111 or vSB1-004. Highest protection rates against morbidity and mortality were obtained in the groups vaccinated with vHVT110 or vHVT114 whatever the challenge strain used (homologous strain i.e. Malaysian genotype VIIId or heterologous strain i.e. Chimalhuacan genotype V). There was a correlation between the % of birds positive by HI test before challenge and the % protection.

The difference of protection obtained between vHVT110 and vHVT111 clearly illustrates the importance of the promoter, the mCMV IE promoter being more potent than the SV40 promoter for the transcription of the wild type (wt) genotype VIIId F gene. The difference of protection obtained between vHVT111 and vHVT114 illustrates the importance of the nucleotide sequence of the F gene, the optimized sequence being more potent than the wild type (or native) sequence.

In conclusion, the results of this study showed the importance of the promoter and the nucleotide sequence of the F gene in the ND protection induced by Marek's disease vector vaccines. An optimal combination of these factors needs to be found to reach the best efficacy performances as for vHVT114.

Example 7

Efficacy of vHVT114, vHVT116, vHVT301, vHVT302 and vHVT303 Expressing the NDV F Gene Against Challenges with NDV Texas GB Strain at 14 Days of Age in SPF Chickens

The aim of the study was to assess the efficacy of 2 single HVT recombinant constructs (vHVT114 and vHVT116)

expressing the NDV F gene and 3 double HVT recombinant constructs (vHVT-301, vHVT302 and vHVT303) expressing both NDV F and IBDV VP2 genes against Newcastle disease challenge (Texas GB strain, genotype II) performed at 14 days of age in SPF chickens.

The characteristics of these 4 vaccine candidates are described in Table 12 below.

TABLE 12

Characteristics of the vectors used in the challenge study					
Name	Parental virus	Promoter	F gene	Poly-A	Locus
vHVT114	HVT	SV40	Opt-VIId	SV40	IG1
vHVT116	HVT	SV40	Opt-V	SV40	IG1
vHVT301	vHVT13*	SV40	Wt-VIId	SV40	IG2
vHVT302	vHVT13	US10	Opt-VIId	US10	US10
vHVT303	vHVT13	US10	Opt-V	US10	US10

*vHVT13 is the active ingredient of the licensed Vaxxitek HVT-IBD vaccine based on an HVT vector expressing the IBDVVP2 gene (see U.S. Pat. No. 5,980,906 and EP 0 719 864).

On D0, 120 one-day-old SPF chickens were randomly allocated into 6 groups of 20 birds. The birds were injected by subcutaneous injection in the neck at D0 with 0.2 mL of recombinant vaccines containing a target dose of 1000 pfu as described in Table 13 below. The birds were challenged by the intramuscular route on D14 with 4.5 log 10 EID50 velogenic ND Texas GB (genotype II) strain.

TABLE 13

Results of efficacy		
Group	Vaccine at day-old (D0)	% clinical protection (number infected/total) after Newcastle challenge at 14 days of age (D14)
G1	—	0% (20/20)
G2	vHVT114	80% (4/20)
G3	vHVT116	70% (6/20)
G4	vHVT301	15% (17/20)
G5	vHVT302	52.6% (9/19)*
G6	vHVT303	15% (17/20)

*1 bird died before challenge

Each group was monitored before and after challenge. NDV clinical signs and mortality were recorded after challenge.

Percentages of clinical protection are reported in the table above. Full susceptibility was observed in the non-vaccinated challenged control group G1 thus validating the high severity of both challenges. Partial protection was observed for the 5 vaccine candidates, the best performances being obtained with vHVT114 and vHVT116. Among the double HVT recombinants, the vHVT302 was the most protective. It performed better than vHVT303 suggesting that the optimized genotype VIId NDV F gene may be better cross-protective against genotype II challenge than the optimized genotype V NDV F gene. A similar tendency was observed with single HVT, the vHVT114 (VIId gene) performing slightly better than vHVT116 (V gene) but the difference was less pronounced. These results indicated that both genotypes VIId and V NDV F genes inserted in the HVT vector provide cross-protection against a heterologous genotype II NDV challenge; the VIId gene may potentially be more cross-protective. The vHVT302 induced a better ND protection than vHVT301 confirming the importance of the promoter, poly-A and locus of insertion. In conclusion, the results of this

study showed the very good early ND protection induced by tested Marek's disease vector vaccines, especially for the tested single HVT-ND.

Example 8

Efficacy of vHVT114, vHVT116, vSB1-007, vSB1-008 (Alone or with vHVT13) and vHVT 304 Against Challenges with NDV ZJ1 (Genotype VIId) and California/02 (Genotype V) at 21 Days of Age in SPF Chickens

The aim of the study was to assess the efficacy of 2 single HVT recombinant constructs (vHVT114 and vHVT116), 2 SB1 recombinant constructs (vSB1-007 & vSB1-008) expressing the NDV F gene and a double HVT recombinant (vHVT304) against Newcastle disease challenge with NDV ZJ1 (genotype VIId) and California/02 (genotype V) performed at 21 days of age in SPF chickens.

The characteristics of these 5 vaccine candidates are described in Table 14 below.

TABLE 14

Characteristics of the vectors used in the challenge study					
Name	Parental virus	Promoter	F gene	Poly-A	Locus
vHVT114	HVT	SV40	Opt-VIId	SV40	IG1
vHVT116	HVT	SV40	Opt-V	SV40	IG1
vSB1-007	SB-1	SV40	Opt-VIId	gC	UL44 (gC)
vSB1-008	SB-1	SV40	Opt-V	SV40	IG1
vHVT304	vHVT13*	SV40	Opt-VIId	Synth	IG2

*vHVT13 is the active ingredient of the licensed Vaxxitek HVT-IBD vaccine based on an HVT vector expressing the IBDVVP2 gene (see U.S. Pat. No. 5,980,906 and EP 0 719 864).

On D0, 158 one-day-old SPF chickens were randomly allocated into 6 groups of 24 birds (vaccinated) and 1 group of 12 birds (non-vaccinated controls). The birds were injected by subcutaneous injection in the neck at D0 with 0.2 mL of recombinant vaccines containing a target dose of 1000 pfu as described in Table 15 below. The birds were then separated into two sub-groups, each sub-group being challenged by the intramuscular route on D21 with 5 log 10 EID50 of either NDV ZJ1 (genotype VIId) or California/02 (genotype V) velogenic strain.

TABLE 15

Results of efficacy			
Group	Vaccine at day-old (D0)	% clinical protection	
		CA/02 (genotype V)	ZJ1 (genotype VIId)
G1	—	0%	0%
G2	vHVT114	100%	100%
G3	vHVT116	100%	90%
G4	vSB1-007	92%	100%
G5	vSB1-008	100%	100%
G6	vSB1-008 + vHVT13	100%	83%
G7	vHVT304	92%	75%

Each group was monitored before and after challenge. Technical problems observed with isolators reduced the number of birds in group 2 (vHVT114: from 24 to 14) and in group 3 (vHVT116: from 24 to 20). NDV clinical signs were recorded after challenge. Serum was collected from blood

samples taken from birds of groups 2 and 7 before challenge (D21) for NDV serology by HI test using each challenge strains as antigen.

Mean serologic HI titers in G2 and G7 before challenge are shown in FIG. 10. HI titers were higher with the ZJ1 antigen in both groups. The HI titers induced by vHVT114 were higher than those induced by vHVT304.

Percentages of protection against mortality and morbidity are reported in the table above. Full susceptibility was observed in the non-vaccinated challenged control group G1 thus validating the high severity of both challenges. All vaccines induced high levels ($\geq 75\%$) of protection against both challenges. Full clinical protection against both challenges was induced by vHVT114 and vSB1-008. Following a similar tendency as the HI titers, the ND protection induced by vHVT304 was slightly lower than that induced by vHVT114.

The shedding was evaluated after challenge by real time RT-PCR in oral and cloacal swabs taken 2 and 4 days post-challenge. Percentage of positive ($Ct < 40$) birds are shown for both challenges in FIGS. 11A and 11B. Note that all 6 birds were dead at 4 dpch in the control group challenged with the CA/02 isolate and only one bird (out of 6) was still alive at 4 dpch in the control group challenged with ZJ1. Shedding was detected in all control birds. Reduction of the percentage of birds positive for shedding was observed in all vaccinated groups.

In conclusion, the results of this study showed the very good ND protection at 3 weeks of age induced by tested Marek's disease vector vaccines.

Example 9

Efficacy of vHVT114, vSB1-007, vSB1-009,
vHVT306 and vHVT307 Vaccines Against
Challenges with NDV Texas GB Strain at 28 Days of
Age in SPF Chickens

The aim of the study was to assess the efficacy of combinations of different Marek's disease vector vaccines expressing the NDV F and/or the IBDV VP2 gene against Newcastle disease challenge (Texas GB strain, genotype II) performed at 28 days of age in SPF chickens.

The characteristics of the 5 recombinant vaccine candidates tested in this study are described in Table 16 below.

TABLE 16

Characteristics of the vectors used in the challenge study					
Name	Parental virus	Promoter	F gene	Poly-A	Locus
vHVT114	HVT	SV40	Opt-VIId	SV40	IG1
vSB1-007	SB-1	SV40	Opt-VIId	gC	UL44 (gC)
vSB1-009	SB-1	SV40	Opt-V	gC	UL44 (gC)
vHVT306	vHVT13	SV40	Opt-VIId	Synth	SORF3-US2
vHVT307	vHVT13	SV40	Opt-V	Synth	SORF3-US2

The Marek's disease virus serotype 1 (CVI988 (or Rispens) strain; Gallid herpesvirus 2) and serotype 2 (SB-1 strain; gallid herpesvirus 3) vaccines were used also in combination with recombinant viruses in some of the groups.

On D0, 135 one-day-old SPF chickens were randomly allocated into 9 groups of 15 birds. The birds were injected by subcutaneous injection in the neck at D0 with 0.2 mL containing a target dose of 2000 pfu for recombinant vaccines (vSB1-007, vSB1-009, vHVT13, vHVT306, vHVT307, vHVT114), and 1000 pfu for parental Marek's disease vac-

cine strains (SB-1 and CVI988). The design of the 9 groups is shown in Table 17 below. The birds were challenged by the intramuscular route on D28 with 4.0 log 10 EID50 velogenic ND Texas GB (genotype II) strain.

TABLE 17

Group	Vaccine at day-old (D0)	Results of efficacy	
		% ND protection after Newcastle disease challenge at 28 days of age	10
G1	—	0%	
G2	vSB1-007 + vHVT13	80%	
G3	vSB1-009	100%	
G4	vSB1-009 + vHVT13	86%	15
G5	vSB1-009 + vHVT13 + CVI988	93%	
G6	vHVT306 + SB-1	100%	
G7	vHVT307	100%	
G8	vHVT307 + SB-1	93%	20
G9	vHVT114 + vHVT13 + SB-1	100%	

Each group was monitored before and after challenge. NDV clinical signs after challenge were recorded.

Percentages of protection against mortality and morbidity are reported in the table above. Full susceptibility was observed in the non-vaccinated challenged control group G1 thus validating the high severity of challenge. Excellent levels of protection were observed in all vaccinated groups. Birds from G3, G6, G7 and G9 were fully protected. This study shows that the vSB1-ND candidates can be co-administered with vHVT13 and CVI988 and still provide a very good ND protection. Similarly, double HVT-IBD+ND are compatible with SB-1 and vHVT-ND (vHVT114) is compatible with vHVT13 and SB-1.

In conclusion, the results of this study showed the lack of interference on ND protection induced by the tested Marek's disease parental and vector vaccines.

Example 10

Efficacy of vHVT114, vHVT307, vSB1-007 and
vSB1-009 in Combination with vHVT13 Against
Challenges with NDV Chimalhuacan Strain
(Genotype V) at D28 in SPF Chickens

The aim of the study was to assess the efficacy of 1 HVT recombinant construct (vHVT114) and 2 SB 1 recombinant constructs (vSB1-007 and vSB1-009) expressing the NDV F gene in combination with vHVT-IBD (vHVT13), as well as a double HVT vHVT307 expressing both NDV F and IBDV VP2 against Newcastle disease challenge (Chimalhuacan, genotype V) performed at 28 days of age in SPF chickens.

The characteristics of these 4 vaccine candidates are described in Table 18 below.

TABLE 18

Characteristics of the vectors used in the challenge study					
Name	Parental virus	Promoter	F gene	Poly-A	Locus
vHVT114	HVT	SV40	Opt-VIId	SV40	IG1
vSB1-007	SB-1	SV40	Opt-VIId	gC	UL44 (gC)
vSB1-009	SB-1	SV40	Opt-V	gC	UL44 (gC)
vHVT307	vHVT13	SV40	Opt-V	Synth	SORF3-US2

On D0, 45 one-day-old SPF chickens were randomly allocated into 4 groups of 10 birds and 1 group of 5 birds (unvaccinated control group). The birds were injected by subcutaneous injection in the neck at D0 with 0.2 mL of recombinant vaccines containing a target dose of 2000 pfu as described in

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Table 19 below. The birds were challenged by the intramuscular route on D28 with 5.0 log 10 EID50 velogenic Chimalhuacan (genotype V) strain.

TABLE 19

Results of efficacy			
Group	Vaccine at day-old (D0)	% protection against mortality	% protection against morbidity
G1	—	0%	0%
G2	vHVT114 + vHVT13	100%	100%
G3	vHVT307	80%	80%
G4	vSB1-007 + vHVT13	90%	90%
G5	vSB1-009 + vHVT13	90%	90%

Each group was monitored before and after challenge. NDV clinical signs were recorded after challenge. Oropharyngeal swabs were taken in the vaccinated groups at 5 and 7 days post-challenge to evaluate the viral load by real time RT-PCR.

Percentages of protection against mortality and morbidity are reported in the table above. Full susceptibility was observed in the non-vaccinated challenged control group G1 thus validating the high severity of challenge. Very good protection was observed in all 4 vaccinated groups, a full clinical protection being induced by vHVT114+vHVT13.

The percentage of positive birds and the mean shedding titer (expressed as log 10 EID50 equivalent per mL) are shown in FIGS. 12A and 12B. Surprisingly, no shedding was detected in G2 indicating a complete (against both clinical signs and shedding) ND protection induced by vHVT114 even if co-administered with vHVT13, in the tested conditions. The shedding levels detected in the other vaccinated groups were low with a slightly higher level detected in G3 (vHVT307) at 5 days post-infection (pi) only.

In conclusion, this example further illustrates the excellent ND protection induced by double HVT-IBD+ND recombinant or a combination of SB1-ND or HVT-ND and HVT-IBD (vHVT13) recombinant viruses. Contrary to the general belief in the field that a second HVT vaccine (regular HVT vaccines or recombinant HVT vaccines) interferes with the immunity to the foreign genes inserted into the first recombinant HVT vaccine, the present invention showed surprising result that vHVT114 in combination with vHVT13 offered excellent protection against NDV and no interference effect was observed.

Example 11

Efficacy of vHVT306, vSB1-008 in Combination with vHVT13 Administered by SC or in Ovo Route Against Challenge with NDV Chimalhuacan Strain (Genotype V) at D28 in SPF Chickens

The aim of the study was to assess the efficacy of the vHVT306 double HVT expressing both NDV F and IBDV VP2 genes, and the vSB1-008 SB1 recombinant expressing the NDV F gene in combination with vHVT-IBD (vHVT13), administered by the in ovo or by the subcutaneous route against Newcastle disease challenge (Chimalhuacan, genotype V) performed at 28 days of age in SPF chickens.

The characteristics of these 2 ND vaccine candidates are reported in the table 14 (vSB1-008) and in table 16 (vHVT306).

The design of the groups is shown on Table 20. Sixty SPF embryonated eggs (after approximately 18 days and 18 hours of incubation; D-3) were used for the in ovo administration

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(20 per group for G1, G2 and G3). Fifty microliters of vaccine containing 2000 PFU were administered by the in ovo route using the IntelliLab System device from AviTech LLC (Salisbury, Md., USA). Hatchability and survival were recorded after in ovo administration. On D0, 20 one-day-old SPF chickens were randomly allocated into 2 groups of 10 birds (G4 and G5). The birds were injected by subcutaneous (SC) injection in the neck at D0 with 0.2 mL of recombinant vaccines containing a target dose of 2000 pfu as described in Table 20 below. Ten birds per group were challenged by the intramuscular route on D28 with 5.0 log 10 EID50 velogenic Chimalhuacan (genotype V) strain.

TABLE 20

Study design and results of ND efficacy				
Group	Vaccine at day-old (D0)	Admin. route	% protection against mortality	% protection against morbidity
G1	vHVT13	In ovo	0%	0%
G2	vHVT306	In ovo	100%	100%
G3	vSB1-008 + vHVT13	In ovo	78%	68%
G4	vHVT306	SC	100%	100%
G5	vSB1-008 + vHVT13	SC	100%	70%

Each group was monitored before and after challenge. NDV clinical signs were recorded after challenge. Oropharyngeal swabs were taken in the vaccinated groups at 5 and 7 days post-challenge to evaluate the viral load by real time RT-PCR.

Full hatchability and viability were recorded up to D28 (challenge day) for birds of groups G1 and G2. Hatchability in G3 was 85% and one additional bird died after hatching in this group. The lower hatchability of that group may be due to egg incubator problems. Body weights of males and females in G1, G2 and G3 were similar at D1 and at D28.

Percentages of protection against mortality and morbidity are reported in the table 20. Full susceptibility was observed in the non-vaccinated challenged control group G1 thus validating the high severity of challenge. Very good protection was observed in all 4 vaccinated groups, a full clinical protection being induced by vHVT306 administered by both routes.

The percentage of positive birds and the mean shedding titer (expressed as log 10 EID50 equivalent per mL) are shown in Table 21. Absence of detectable or very low shedding was observed in G2 and G4 vaccinated with vHVT306. The shedding levels detected in the groups vaccinated with vSB1-008+vHVT13 were higher especially at 5 days post-infection (pi).

TABLE 21

Results of protection against shedding (percentage of birds with detectable shedding and mean viral load in log10) evaluated at D5 and D7 after NDV challenge				
Group	Vaccine at day-old (D0)	Admin. Route	Percent of positive birds (D5/D7 pi)	Mean viral load* (D5/D7 pi)
G2	vHVT306	In ovo	0/0%	2.7/2.7
G3	vSB1-008 + vHVT13	In ovo	100/38%	5.2/3.2
G4	vHVT306	SC	20/10%	3.2/2.9
G5	vSB1-008 + vHVT13	SC	80/50%	4.6/3.4

*Mean quantitative real time PCR value expressed in equivalent log10 EID50; the threshold is set at 2.7 log10.

In conclusion, this example shows excellent ND protection induced by vHVT306 double HVT recombinant adminis-

tered either by in ovo or by SC routes. The performance of vSB1-008+vHVT13 was slightly lower especially after in ovo administration, but it may be at least partially due to egg incubator problems. Indeed, the in ovo safety testing of another SB1-ND recombinant (vSB1-009) at 1000 or 4000 PFU associated with 6000 PFU of vHVT13 did not show any difference in hatchability and early survival with a group receiving 6000 PFU of vHVT13 only.

Example 12

Efficacy of vHVT304, vHVT306, vSB1-007 and vSB1-008 in Combination with vHVT13 Against Challenge with NDV Chimalhuacan Strain (Genotype V) at D42 in Commercial Broiler Chickens

The aim of the study was to assess the efficacy of two double HVT (vHVT304 and vHVT306) expressing both NDV F and IBDV VP2 genes, and two SB1 recombinants (vSB1-007 and vSB1-008) expressing the NDV F gene in combination with vHVT-IBD (vHVT13) against Newcastle disease challenge (Chimalhuacan, genotype V) performed at 42 days of age in commercial broiler chickens.

The characteristics of these 4 ND vaccine candidates are reported in tables 14 and 16. The design of the groups is shown on Table 22. On D0, 55 one-day-old commercial broiler chickens were randomly allocated into 5 groups of 11 birds. The birds were injected by subcutaneous (SC) injection in the neck at D0 with 0.2 mL of recombinant vaccines containing a target dose of 2000 pfu as described in Table 22 below. Ten birds per group were challenged by the intramuscular route on D42 with 5.0 log 10 EID50 velogenic Chimalhuacan (genotype V) strain.

TABLE 22

Study design and results of ND efficacy			
Group	Vaccine at day-old (D0)	% protection against mortality	% protection against morbidity
G1	vHVT13	0%	0%
G2	vHVT304	82%	82%
G3	vHVT306	100%	100%
G4	vSB1-007 + vHVT13	100%	100%
G5	vSB1-008 + vHVT13	91%	91%

Each group was monitored before and after challenge. NDV clinical signs were recorded during 14 days after challenge. Oropharyngeal swabs were taken in the vaccinated groups at 5 and 7 days post-challenge to evaluate the viral load by real time RT-PCR.

Percentages of protection against mortality and morbidity are reported in the table 22. Full susceptibility was observed in the non-vaccinated challenged control group G1 thus validating the high severity of challenge. Very good protection was observed in all 4 vaccinated groups, a full clinical protection being induced by vHVT306 and by vSB1-007+vHVT13.

The percentage of positive birds and the mean shedding titer (expressed as log 10 EID50 equivalent per mL) are shown in Table 23. The best reduction of shedding was induced by vHVT306 and vSB1-007+vHVT13, which were also the best candidates for clinical protection.

TABLE 23

Results of protection against shedding (percentage of birds with detectable shedding and mean viral load in log10) evaluated at D5 and D7 after NDV challenge (pi)			
Group	Vaccine at day-old (D0)	Percent of positive birds (D5/D7 pi)	Mean viral load* (D5/D7 pi)
G2	vHVT304	100/100%	5.4/4.6
G3	vHVT306	40/50%	3.5/3.7
G4	vSB1-007 + vHVT13	80/70%	3.8/4.8
G5	vSB1-008 + vHVT13	100/100%	4.8/4.3

*Mean quantitative real time PCR value expressed in equivalent log10 EID50; the threshold was set at 2.7 log10.

The vHVT306 ND protection was found to be better than that of vHVT304. These two double HVT contain the same NDV F expression cassette but inserted in two different loci, the IBDV VP2 one being inserted at the same position. This example therefore illustrates the importance of the locus of insertion in the design of HVT recombinants. The vSB1-007+vHVT13 was better than vSB1-008+vHVT13. The vSB1-007 genomic structure differs from that of vSB1-008 in different aspects: locus of insertion, promoter, poly-adenylation signal and F gene origin. The combination of these foreign sequences and locus of insertion in vSB1-007 were likely responsible for its better ND protection performances.

In summary, this example illustrates the importance of the locus of insertion and other regulatory sequences of the NDV expression cassette in the ND protection induced by HVT and MDV serotype 2 vectors.

Example 13

Efficacy of Double HVT-ND+IBD (vHVT304 and vHVT306) or SB1-ND (vSB1-008) in Combination with vHVT13 Recombinant Vaccines, Against Challenge with a Classical IBDV Isolate on D14 in SPF Chickens

The aim of the study was to assess the early IBD efficacy of double HVT recombinants vHVT304 and vHVT306 as well as that of vHVT13 co-administered with a SB1-ND (vSB1-008) recombinant constructs against a virulent infectious bursal disease virus (vIBDV) challenge (Faragher 52/70 strain) performed at 14 days of age in SPF chickens.

The characteristics of the double HVT and SB1 recombinants used in this study are shown in Tables 14 and 16.

On D0, 95 one-day-old SPF chickens were randomly allocated into 9 groups of 10 birds and 1 group of 5 birds (unvaccinated unchallenged control group). The birds were injected by subcutaneous injection in the neck at D0 with 0.2 mL of recombinant vaccines containing a target dose of 300 or 1000 pfu as described in the Table 24 below. On D14, blood sample was collected from 5 birds per group for serological testing with the Kit ProFLOK® plus IBD (Synbiotics Corp). The birds (10 birds per group except for group 7 in which 1 bird died before challenge) were challenged by the eye drop (0.05 mL per bird) on D14 with 2.5 log 10 EID50.

TABLE 24

Study design and results of IBD efficacy					
Group	Vaccine at day-old (dose in PFU)	IBD+ ELISA titer at D14 ¹	Number Dead/ Sick ²	% protection ³	Mean bursal/body weight ratio ⁴
G1	vSB1-008 (1000)	0.2	7/10	0%	0.0013
G2	vHVT13 (300)	2.7	0/0	100%	0.0051
G3	vHVT13 (1000)	2.7	0/0	90%	0.0049
G4	vHVT13 + vSB1-008 (300)	1.9	1/1	60%	0.0041
G5	vHVT13 + vSB1-008 (1000)	2.4	0/0	70%	0.0041
G6	vHVT304 (300)	2.9	0/0	60%	0.0037
G7	vHVT304 (1000)	2.2	0/0	67%	0.0047
G8	vHVT306 (300)	2.4	0/0	80%	0.0033
G9	vHVT306 (1000)	2.7	0/0	40%	0.0026

¹Mean IBD+ ELISA titers expressed in log10 in the serum of 5 birds per group sampled at D14 before challenge;

²Birds sick for more than 2 days or still sick on D25 were considered as sick.

³Protection against clinical signs and severe bursal lesion (bursal score <3)

⁴The bursal/body weight ratio of the unvaccinated/unchallenged group was 0.0047.

Each group was monitored before and after challenge. IBDV clinical signs were recorded for 11 days after challenge (from D15 to D25). At the end of the post-challenge observation period (D33), all the surviving birds were euthanized and necropsied. Body and bursal weights were recorded. Each bursa of Fabricius (BF) was weighted then stored in individual recipients containing 4% formaldehyde for histology. Histological lesions of the bursa were scored according to the scale presented in Table 25.

TABLE 25

Score	Histology observation/lesions
0	No lesion, normal bursa
1	1% to 25% of the follicles show lymphoid depletion (i.e. less than 50% of depletion in 1 affected follicle), influx of heterophils in lesions
2	26% to 50% of the follicles show nearly complete lymphoid depletion (i.e. more than 75% of depletion in 1 affected follicle), affected follicles show necrosis and severe influx of heterophils may be detected
3	51% to 75% of the follicles show lymphoid depletion; affected follicles show necrosis lesions and a severe influx of heterophils is detected
4	76% to 100% of the follicles show nearly complete lymphoid depletion; hyperplasia and cyst structures are detected; affected follicles show necrosis and severe influx of heterophils is detected
5	100% of the follicles show nearly complete lymphoid depletion; complete loss of follicular structure, thickened and folded epithelium, fibrosis of bursal tissue

*sourced from Monograph No. 01/2008:0587 of EU Pharmacopoeia "Avian Infectious Bursal Disease vaccine (live)"

A bird was considered as affected if it died and/or showed notable sign of disease and/or severe lesions of the bursa of Fabricius (i.e., histology score 3).

The mean ELISA IBD+ antibody titer expressed in log 10 before challenge is shown in Table 24. Significant titers were detected in all vaccinated groups that were significantly higher than that of the control group G1. The serology titer was not dose-dependent.

Severe clinical signs were observed after challenge in all birds of the control group G1. Seven out of 10 birds of that group died within the 11 days observation period indicating the high severity of challenge. None of the vaccinated birds showed severe clinical signs after challenge except 1 bird of G4 that died. Percentages of protection against severe bursal lesions are shown in the table above. Significant IBD protec-

tion was observed in all groups, the best protection being observed in G2 and G3 (vHVT13 alone). The co-administration of vSB1-008+vHVT13 and the double vHVT304 and vHVT306 constructs induced similar levels of IBD protection. The protection was not dose-dependent at the tested doses. The mean bursal/body weight ratios are also shown in Table 24. Ratios in all vaccinated groups were higher than those of the challenged control group.

In conclusion, these data indicate that both the combination of a SB1-ND vector with a single HVT-IBD or double HVT expressing both NDV-F and IBDV-VP2 induce IBD antibodies and early IBD protection in a severe IBDV challenge model.

Example 14

Efficacy of Single HVT-ND (vHVT114) or SB1-ND (vSB1-007 and vSB1-009) in Combination with vHVT13 Recombinant Vaccines, Against Challenge with a Very Virulent IBDV Isolate on D23 in Commercial Broiler Chickens

The aim of the study was to assess the IBD efficacy of vHVT13 co-administered with an HVT-ND (vHVT114) or SB1-ND (vSB1-007 and vSB1-009) recombinant constructs against a very virulent infectious bursal disease virus (vvIBDV) challenge (91-168/980702) performed at 23 days of age in commercial broiler chickens.

The characteristics of these 4 vaccine candidates are described in Tables 14 and 16. On D0, 90 one-day-old broiler chickens were randomly allocated into 7 groups of 12 birds and 1 group of 6 birds (unvaccinated unchallenged control group). The birds were injected by subcutaneous injection in

the neck at D0 with 0.2 mL of recombinant vaccines containing a target dose of 3000 pfu as described in the Table 26. On D14, blood sample was collected from 5 birds per group for serological testing with the Kit ProFLOK® plus IBD (Synbiotics Corp). The serum of 10 extra one-day-old broiler chickens was tested at D0 with the same kit to evaluate the level of IBDV maternal antibody. The birds (10 birds per group) were challenged by the eye drop (0.05 mL per bird) on D23 with 4.3 log 10 EID50 of the vvIBDV 91-168 isolate.

Each group was monitored before and after challenge. IBDV clinical signs were recorded for 11 days after challenge (from D23 to D33). At the end of the post-challenge observation period (D33), all the surviving birds were euthanized

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and necropsied. Body and bursal weights were recorded. Each bursa of Fabricius (BF) was weighted then stored in individual recipients containing 4% formaldehyde for histology. Histological lesions of the bursa were scored according to the scale presented in Table 25.

A bird was considered as affected if it died and/or showed notable signs of disease and/or severe lesions of the bursa of Fabricius (i.e., histology score ≥ 3).

TABLE 26

Study design and serology results			
Group	Vaccine at day-old (D0)	IBD+ ELISA titer at D23 ¹	Mean bursal/body weight ratio ²
G1	—	3.9	0.0007
G2	vHVT13	4.0	0.0015
G3	vHVT114 + vHVT13	4.1	0.0015
G4	vSB1-007 + vHVT13	3.8	0.0018
G5	vSB1-009 + vHVT13	4.0	0.0019

¹Mean IBD+ ELISA titers expressed in log10 in the serum of 5 birds per group sampled at D23 before challenge;

²The bursal/body weight ratio of the unvaccinated/unchallenged group was 0.0047

The mean ELISA IBD+ serological titer at D0 was $4.36 \pm 0.01 \log 10$ indicating a very high level of IBD maternal antibody at hatch. At D23, the mean ELISA IBD+ titer was still high (3.9) in the control G1. ELISA mean titers in the vaccinated groups were not significantly different from those of the control group.

Neither morbidity nor mortality was observed in any of the groups after challenge. Percentages of protection against severe bursal lesions are shown in the table 26 above. The result showed that co-administration of vHVT114, vSB1-007 or vSB1-009 did not interfere with vHVT13-induced IBD protection indicating a lack of interference. Similarly, the mean bursal/body weight ratios of the vaccinated groups were similar and clearly higher than that of the control group, indicating IBD protection and no difference between the vaccination regimens.

In conclusion, the data indicate the compatibility between vHVT114, vSB1-007 or vSB1-009 and vHVT13 for IBD protection. The lack of interference between the two HVT vectors for IBD protection was again surprising and confirmed the results observed for ND protection (see example 10),

Example 15

Efficacy of Double HVT-ND+IBD (vHVT304 and vHVT306) Associated or not with SB-1 and of SB1-ND (vSB1-007 and vSB1-008) in Combination with vHVT13 Recombinant Vaccines, Against Challenge with a Variant E IBDV Isolate on D28 in SPF Chickens

The aim of the study was to assess the efficacy of two double HVT (HVT-ND+IBD: vHVT304 and vHVT306) or two vSB1-NDV in combination with vHVT13 (vSB1-007+vHVT13, vSB1-008+vHVT13) vectored vaccines administered subcutaneously (SC) to day-old SPF chicks and challenged with IBDV-Variant (VAR-E) 28 days post-vaccination.

On D0, 105 one-day-old SPF chickens were randomly allocated into 7 groups of 15 birds including a group of challenged controls (G6) and unchallenged controls (G7). The birds of groups G1 to G5 were injected by subcutaneous

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injection in the neck at D0 with 0.2 mL of recombinant and/or SB-1 vaccines containing each a target dose of 2000 pfu. The design of the study is shown in Table 27 below. On D28, all birds from groups G1 to G6 were challenged by the eye drop (0.03 mL containing $3 \log 10$ EID50 per bird) of the IBDV variant E isolate from University of Delaware (USA). Each group was monitored before and after challenge. Eleven days post-challenge, birds were weighed and necropsied. The bursa were collected and weighed. The bursal/body weight ratios (bursa weight/body weight ratio $\times 100$) were calculated.

TABLE 27

Study design and results of IBD efficacy		
Group	Vaccine at day-old	Mean bursal/body weight ratio (*100)
G1	vHVT304	0.33
G2	vHVT304 + SB-1	0.33
G3	vHVT306	0.29
G4	vHVT13 + vSB1-007	0.49
G5	vHVT13 + vSB1-008	0.47
G6	- (challenged)	0.13
G7	- (unchallenged)	0.46

The mean bursal/body weight ratios are shown in the Table 27. The challenged control birds had a severe bursal atrophy compared to unchallenged ones. The vSB1-007 and vSB1-008 vaccines did not interfere on vHVT13-induced protection (G4 and G5). The bursal/body weight ratios of birds vaccinated with the double HVT (HVT-ND+IBD) were slightly lower than the unchallenged control group but were clearly higher than the challenged control groups. Furthermore, the SB-1 serotype 2 Marek's disease vaccine did not interfere with vHVT304-induced IBD protection.

In conclusion, these data indicate that both the combination of a SB1-ND vector with a single HVT-IBD or double HVT expressing both NDV-F and IBDV-VP2 induce IBD protection in a variant E IBDV challenge model.

Example 16

Lack of Interference of vHVT114, vSB1-009 and/or SB-1 on vHVT13 Induced Variant E IBD Protection in SPF Chickens

The aim of the study was to assess the IBD efficacy of vHVT13 when administered by SC or in ovo route concomitantly with vHVT114, vSB1-009 and/or SB-1 in SPF chicks in an IBDV-variant (VAR-E) at D28 challenge model.

75 one-day-old SPF chickens and 75 SPF 18 to 19 day-old chicken embryo were randomly allocated into 5 groups (G1 to G5 and G6 to G10, respectively) including a group of challenged controls (G4 and G9, respectively) and unchallenged controls (G5 and G10, respectively). The birds of groups G1 to G3 were injected by subcutaneous injection in the neck at D0 with 0.2 mL of vaccines containing each a target dose of 3000 pfu except for SB-1 which had a target dose of 1000 PFU. Birds from G6 to G8 received the same vaccine doses but in 0.05 mL volume by the in ovo route 2-3 days before hatch. The design of the study is shown in Table 28 below. At 28 days of age, all birds from groups G1 to G4 and G6 to G9 were challenged by the eye drop (0.03 mL containing $3 \log 10$ EID50 per bird) of the IBDV variant E isolate from University of Delaware (USA). Each group was monitored before and after challenge. Eleven days post-challenge, birds were weighed and necropsied. The bursa were collected and

weighed. The bursal/body weight ratios (bursa weight/body weight ratio $\times 100$) were calculated.

TABLE 28

Study design and results of IBD efficacy			
Group	Vaccine at day-old	Administration route	Mean bursal/body weight ratio (*100)
G1	vHVT13 + vHVT114 + SB-1	SC	0.56
G2	vHVT13 + vHVT114 + vSB1-009	SC	0.58
G3	vHVT13 + vSB1-009	SC	0.52
G4	- (challenged)	SC	0.13
G5	- (unchallenged)	SC	0.51
G6	vHVT13 + vHVT114 + SB-1	In ovo	0.54
G7	vHVT13 + vHVT114 + vSB1-009	In ovo	0.47
G8	vHVT13 + vSB1-009	In ovo	0.53
G9	- (challenged)	In ovo	0.14
G10	- (unchallenged)	In ovo	0.58

The mean bursal/body weight ratios are shown in the Table 28. The challenged control birds (G4 and G9) had a severe bursal atrophy compared to unchallenged ones. The bursal/body weight ratios of the vaccinated groups (G1 to G3 and G6 to G8) were similar to those of the unchallenged control groups (G5 and G10) and well above those of the challenged control groups (G4 and G9). The lack of interference of vHVT114 on vHVT13-induced IBD protection after both SC or in ovo routes was surprising and confirmed data obtained in examples 10 and 14.

In conclusion, these data indicate clearly the compatibility of vHVT114+vSB1-009 or +SB-1 and of vSB1-009 with vHVT13 when administered by SC or in ovo route in a variant E IBDV challenge model.

Example 17

Efficacy of vHVT114 and vHVT13 and SB1 or vSB1-009 Vectors Against Very Virulent Plus Marek's Disease Challenge

The aim of this study was to evaluate the Marek's disease efficacy induced by different combinations of vaccines including vHVT114, vHVT13, SB-1 and/or vSB1-009 administered by the SC route to one-day-old SPF chicks and challenged 4 days later with the very virulent plus Marek's disease virus (vv+MDV) T-King isolate.

On D0, 100 one-day-old SPF chickens were randomly allocated into 5 groups of 20 birds. The birds from groups 1 to 3 were injected by subcutaneous injection in the neck at D0 with 0.2 mL of vaccines containing a target dose of 2000 pfu for each vaccine except for SB-1 for which the target dose was 1000 pfu. Birds from groups 4 and 5 were non-vaccinated and were used as sham controls challenged (group 4) or unchallenged (group 5). The study design is shown in the Table 29. On D4, All birds from groups 1 to 4 were challenged with 0.2 mL of the vv+MDV T-King isolate using the intraperitoneal route of administration.

TABLE 29

Study design and MD protection results			
Group	Vaccine at day-old (D0)	Number of MD positive/total	Percentage of protection
G1	vHVT13 + SB-1	7/20	65%
G2	vHVT114 + SB-1	7/20	65%
G3	vHVT13 + vHVT114 + vSB1-009	7/20	65%
G4	- (challenged)	20/20	0%
G5	- (unchallenged)	0/20	100%

Each group was monitored daily for any unfavourable reactions before and after challenge. At day 49, all live birds were terminated and necropsied to examine for gross lesions associated with Marek's disease. Chickens were classified as positive for infection with Marek's disease if nervous signs, such as paralysis, locomotive signs attributable to the disease, and severe emaciation or depression are observed, if mortality directly attributable to Marek's Disease occurs, or if gross lesions are observed at necropsy. Lesions might include, but not be limited to, the following: liver, heart, spleen, gonads, kidneys, and muscle lesions.

Results of protection are shown in the Table 29 above. All vaccinated groups (G1 to G3) performed equally, inducing a partial (65%) MD protection as expected in this very severe and early challenge model. These results indicated that the vector vaccine candidates retain their ability to protect against Marek's disease.

Example 18

Efficacy of Recombinant HVT and SB1 Vectors Against Marek's Disease

Marek's disease efficacy is also demonstrated for the HVT vectored recombinants and the SB-1 vectored recombinants either alone or in combination. The challenge strains include a virulent Marek's disease (vMD) challenge such as GA22, a very virulent Marek's disease (vvMD) challenge such as RB1B and/or a very virulent plus Marek's disease (vv+MD) challenge such as the T. King virus. One-day-old chickens are inoculated subcutaneously or 18-19-day-old embryonated eggs are inoculated with a 0.2 ml dose or 0.05 ml dose, respectively, of the test viruses. At five days of age the vaccinated chickens and naïve controls are challenged with the relevant Marek's challenge virus (v, vv, or vv+MDV). The challenged birds are observed until seven weeks of age. All birds are terminated and necropsied to observe for grossly visible lesions associated with Marek's disease as described in Example 17.

Example 19

Interference of HVT on vHVT13-Induced IBDV Antibodies in Commercial Pullets

The objective of this study was to determine if co-administration of HVT with vHVT13 had an impact on vHVT13-induced IBDV antibody response in commercial pullets.

Eighty day-old commercial brown pullets were used in three isolation units. Fifteen were blood sampled at day-old to test IBD maternally derived antibodies (MDA). The remaining birds were split into three groups as shown in Table 30. Birds from group 2 and 3 were vaccinated by the SC route in the nape of the neck with commercial doses of vHVT13 (VAXXITEK HVT+IBD; Merial SAS, Lyon, France) and/or

HVT cell-associated Bio HVT (Merial S.p.A., Noventa, Italy). Blood sampling was performed at the age of 25, 35 and 45 days of age. The ELISA kit used to evaluate IBDV serological response was the PROFLOK PLUS IBD (IBD+) Ab ELISA kit from Synbiotics (Synbiotics Corp., Kansas City, Mo., USA).

TABLE 30

Study design and serology results					
Group	Vaccine at day-old (D 0)	ELISA titre D 1	ELISA titre D 25	ELISA titre D 35	ELISA titre D 45
G1	—	10,502	7,814	6,237	3,664
G2	vHVT13	10,502	8,023	9,360	9,486
G3	vHVT13 + HVT	10,502	6,896	4,763	3,795

Mean ELISA titers are shown in Table 30. Titers in the unvaccinated group G1 decreased from D1 to D45, which corresponded to the decline of IBDV maternal antibodies. As expected; ELISA titers in the vHVT13 group G2, remains high up to D45 indicating maternal antibodies were progressively replaced by vHVT13-induced antibodies. The addition of HVT to vHVT13 had a clear negative impact since the antibody titers observed in G3 were similar to G1. These results contrast with those obtained with vHVT114+vHVT13 since the vHVT114 did not decrease vHVT13-induced IBD+ ELISA titers (see example 14, Table 26). They confirm the unexpected property of vHVT114 in not interfering with vHVT13 immunogenicity.

In conclusion, in contrast to what was observed with vHVT114, the addition of HVT to vHVT13 had a clear negative impact on vHVT13-induced IBDV humoral immunity.

Example 20

Interference of Commercial HVT-ND on vHVT13-Induced IBD Protection

The objective of this study was to determine if co-administration of commercial HVT-ND vector vaccines with vHVT13 had an impact on vHVT13-induced IBD protection in SPF chickens.

Seventy five SPF chickens (3 groups (G2, G3 and G4) of 25) were vaccinated at one day-of-age by the SC route with a commercial dose of vHVT13 (VAXXITEK HVT+IBD) with or without one commercial dose of licensed HVT-vectorized ND vaccine (vHVT-ND1 and vHVT-ND2) as shown in the Table 31. Fifteen birds were kept as non-vaccinated controls (G1). Three weeks post-vaccination, birds (20 chickens in G2, G3 and G4 and 10 chickens in G1) were challenged with at least 2.0 log 10 EID50 in 0.05 ml of IBD virus Ph/B1 strain (isolated in the Philippines) administered via ocular route. All chickens were observed for 5 days for clinical signs or death from causes attributable to IBD challenge virus and euthanized humanely at end of post-challenge observation for necropsy examination of IBD lesion, especially from the bursa of Fabricius. Birds were considered as protected if their bursa did not show bursal lesions typical of IBD: bursal atrophy, peri-bursa edema and/or hemorrhages in bursa tissues.

TABLE 31

Study design and IBD protection data					
Group	Vaccine at day-old (D 0)	Number of sick (dead)/total	Number of positive bursa/total	Percent of protection	
G1	—	10(8)/10	10/10	0%	
G2	vHVT13 + vHVT-ND 1	3(3)/20	9/20	55%	
G3	vHVT13 + vHVT-ND2	3(1)/20	7/20	65%	
G4	vHVT13	0(0)/20	0/20	100%	

Results are shown in Table 31. All 10 challenged control birds showed clinical signs and 8 out of 10 died 4 or 5 dpi indicating that the IBDV challenge was very severe. All of them had severe lesions of bursa including severe atrophy and haemorrhagic patches. The vHVT13 alone induced full protection whereas both combinations with vHVT-ND induced partial clinical and bursal protection.

In conclusion, these results clearly indicate that the 2 commercial HVT-vectorized ND vaccines interfere with vHVT13-induced IBD protection.

Example 21

Efficacy of vSB1-004, vSB1-006, vSB1-007, vSB1-008, SB1-Vectored ND Vaccine Alone or in Association with vHVT13 HVT-Vectorized IBD Vaccine, and the vHVT302 and vHVT304 Vaccines Against Challenges with NDV Texas GB Strain at 14 and/or 28 Days of Age in SPF Chickens

The aim of the study was to assess the efficacy of combinations of different Marek's disease vector vaccines expressing the NDV F and/or the IBDV VP2 gene against Newcastle disease challenge (Texas GB strain, genotype II) performed at 14 and/or 28 days of age in SPF chickens.

The characteristics of the 6 NDV recombinant vaccine candidates tested in this study are described in the Table 32 below.

TABLE 32

characteristics of the 6 NDV recombinant vaccine candidates tested in this study					
Name	Parental virus	Promoter	F gene	Poly-A	Locus
vSB1-004	SB-1*	mCMV IE	Wt-VIId	SV40	SORF4/US10
vSB1-006	SB-1	SV40	Opt-VIId	Synthetic	UL55/LORF5
vSB1-007	SB-1	SV40	Opt-VIId	(endogeneous from gC gene)	gC
vSB1-008	SB-1	SV40	Opt-CA02	Synthetic	UL55/LORF5
vHVT302	vHVT13	US10	Opt-VIId	US10	US10
vHVT304	vHVT13	SV40	Opt-VIId	Synthetic	IG2

On D0, 225 one-day-old SPF chickens were randomly allocated into 9 groups of 15 birds (G1a to G9a challenged at D14) and 6 groups of 15 birds (G1b, G3b, G4b, G5b, G8b, G9b challenged at D28). The birds were injected by subcutaneous injection in the neck at D0 with 0.2 mL containing a target dose of 2000 pfu for recombinant vaccines. The design of the study is shown in Table 33 below. The birds were challenged by the intramuscular route on D14 or D28 with 4.3

and 4.2 log 10 EID50 (0.1 mL) velogenic ND Texas GB (genotype II) strain, respectively.

TABLE 33

Group	Results of ND efficacy		
	Vaccine at day-old (D 0)	% ND protection after ND challenge at 14 days of age	% ND protection after ND challenge at 28 days of age
G1a & 1b	—	0%	0%
G2a	vSB1-004	20%	ND*
G3a & 3b	vSB1-006	26.6%	73.3%
G4a & 4b	vSB1-007	33.3%	93.3%
G5a & 5b	vSB1-008	46.6%	86.6%
G6a	vSB1-006 + vHVT13	14%	ND
G7a	vSB1-008 + vHVT13	21.4%	ND
G8a & 8b	vHVT302	13.3%	80%
G9a & 9b	vHVT304	33.3%	93.3%

*ND = not done

Each group was monitored before and after challenge. NDV clinical signs after challenge were recorded. One bird died in G6 and G7 before challenge reducing the number of birds from 15 to 14 in these groups.

Percentages of clinical protection (including protection against both mortality and morbidity) are reported in Table 33 above. Full susceptibility was observed in the non-vaccinated challenged control group G1a and G1b thus validating the high severity of challenge. Partial protections ranging from 13.3 to 46.6% were observed after challenge at D14, the highest levels of protection being induced by vSB1-008, vSB1-007 and vHVT304. Protection levels after ND challenge at D28 were much higher for all vaccinated groups and were again slightly higher in the groups vaccines with vSB1-008, vSB1-007 or vHVT304. These results indicated that ND protection levels were dependent on the date of challenge and on the construct. The vSB1-008 and vSB1-007 constructs performed slightly better than vSB1-004 and vSB1-006, and the vHVT304 performed slightly better than vHVT302, indicating that different characteristics of the constructs are playing a role in the performances of MDV-based vector vaccines.

In conclusion, the results of this study showed that ND protection levels induced by Marek's disease vectors expressing NDV F gene may depend on different parameters including the vector, the locus of insertion, the F gene, the promoter, the poly-adenylation site and the challenge conditions.

Example 22

Efficacy of Double HVT-ND+IBD vHVT304 and vHVT306 Vaccines Against Challenges with NDV Texas GB Strain at 14 and/or 28 Days of Age in SPF Chickens

The aim of the study was to assess the efficacy of HVT-vectorized vaccine expressing both NDV F and IBDV VP2 genes against Newcastle disease challenge (Texas GB strain, genotype II) performed at 14 and/or 28 days of age in SPF chickens.

The characteristics of the 2 recombinant vaccine candidates tested in this study are described in the Table 34 below.

TABLE 34

Characteristics of the recombinant vaccine candidates used in this study					
5	Name	Parental virus	Promoter	F gene	Poly-A Locus
vHVT304	vHVT13	SV40	Opt-VIId	Synthetic	IG2
vHVT306	vHVT13	SV40	Opt-VIId	Synthetic	SORF3-US2

On D0, 90 one-day-old SPF chickens were randomly allocated into 3 groups of 15 birds (G1a to G3a challenged at D14) and 3 groups of 15 birds (G1b to G3b challenged at D28). The birds were injected by subcutaneous injection in the neck at D0 with 0.2 mL containing a target dose of 2000 pfu for recombinant vaccines. The design of the study is shown in Table 35 below. The birds were challenged by the intramuscular route on D14 or D28 with a target dose of 4.0 log 10 EID50 (0.1 mL) velogenic ND Texas GB (genotype II) strain.

TABLE 35

25	Results of ND efficacy			
	Group	Vaccine at day-old (D 0)	% ND protection after ND challenge at 14 days of age	% ND protection after ND challenge at 28 days of age
G1a & 1b	—	—	0%	0%
G2a & 2b	vHVT304	—	26.7%	92.9%
G3a & 3b	vHVT306	—	33.3%	86.7%

Each group was monitored before and after challenge. NDV clinical signs after challenge were recorded. One bird died in G2b before challenge reducing the number of birds from 15 to 14 in this group.

Percentages of clinical protection (including protection against both mortality and morbidity) are reported in Table 35 above. Full susceptibility was observed in the non-vaccinated challenged control group G1a and G1b thus validating the high severity of challenge. Protections levels after challenge at D14 were much lower than those obtained after challenge at D28. These vaccine candidates had the same NDV F expression cassette inserted into 2 different loci of vHVT13 genome. They performed equally in terms of ND protection in the tested conditions, indicating that both insertion loci (IG2 and SORF3-US2) are equally suitable for NDV F cassette insertion.

In conclusion, the results of this study showed that ND protection levels induced by Marek's disease vectors expressing NDV F gene depend on different parameters including the vector, the locus of insertion, the F gene, the promoter, the poly-adenylation site and the challenge conditions.

Example 23

ND Early Efficacy Induced by Double HVT-ND+IBD (vHVT302, vHVT303, and vHVT304) or SB1-Vectors (vSB1-006 and vSB1-007) in One Day-Old SPF Chickens Against a Velogenic Genotype V NDV Challenge

The objective of the study was to evaluate the efficacy of three double HVT-ND+IBD (vHVT302, vHVT303, and vHVT304) and two SB1-ND vectors (vSB1-006 and vSB1-007) in one day-old SPF chickens against a velogenic genotype V (Chimalhuacan) NDV challenge performed at D14.

The characteristics of the 5 recombinant vaccine candidates tested in this study are described in Table 36 below.

TABLE 36

Characteristics of the recombinant vaccine candidates used in this study					
Name	Parental virus	Promoter	F gene	Poly-A	Locus
vHVT302	vHVT13	US10	Opt-VIId	US10	US10
vHVT303	vHVT13	US10	Opt-V (CA02)	US10	US10
vHVT304	vHVT13	SV40	Opt-VIId	Synthetic	IG2
vSB1-006	SB-1	SV40	Opt-VIId	Synthetic	UL55/ LORF5
vSB1-007	SB-1	SV40	Opt-VIId	(endogeneous from gC gene)	gC

Six groups (1 and 2) of ten one-day-old specific pathogen free (SPF) white Leghorn chicks were randomly constituted. Birds from groups 2 to 6 were vaccinated by the subcutaneous route (nape of the neck) with a target dose of 2000 PFU as shown in the Table 37 below. Chickens from group 1 were not vaccinated and were kept as control birds. At 2 week-of-age, all birds were challenged with the genotype V Mexican Chimalhuacan (Mex V) velogenic NDV strain. The challenge was performed by the intramuscular (IM) route using 10^5 Egg Infectious Dose 50 (EID50) diluted in 0.2 ml of physiological sterile water. All birds were monitored until 14 days post-challenge. After challenge, health status of each bird was scored daily as follows: healthy/with specific symptoms (ruffled feathers, prostration, torticollis, tremor)/dead. Any bird that showed specific symptoms for more than 2 days or was noted sick on D28 was taken into account for calculation of morbidity.

TABLE 37

Results of early ND protection induced by different MDV vectored candidates expressing NDV F gene in SPF day-old chicks				
Group	Vaccine	Target dose (PFU) under 0.2 mL (actual dose)	Protection against mortality	Protection against morbidity
G1	—	—	0%	0%
G2	vHVT302	2000 (4427)	50%	10%
G3	vHVT303	2000 (ND)	10%	0%
G4	vHVT304	2000 (1169)	80%	60%
G5	vSB1-006	2000 (1720)	60%	40%
G6	vSB1-007	2000 (1564)	80%	50%

Results of protection are summarized in Table 37. All control birds died after ND challenge. Variable levels of ND protection were induced by the different tested vaccines ranging from 10% to 80% and from 0% and 60% in terms of protection against mortality and morbidity, respectively. The vHVT304 candidate induced a better protection than the vHVT303 and vHVT302 candidates; this may be due to the exogenous SV40 promoter placed in front of the NDV F gene. The vSB1-007 performed slightly better than the vSB1-006. Furthermore, performances obtained with vHVT304 were comparable to those obtained with vSB1-007 indicating that different Marek's disease vectors can reach the same level of ND protection.

In conclusion, this study demonstrates that both double HVT-ND+IBD and SB1-ND vectored vaccines can reach significant levels of ND protection in a very severe and early NDV challenge model.

Example 24

ND Efficacy Induced by the Double HVT-ND+IBD vHVT306 Administered by in Ovo or SC Route to One Day-Old SPF Chickens Against a Velogenic Genotype V NDV Challenge Performed at D28

The objective of the study was to evaluate the efficacy of one double HVT-ND+IBD (vHVT306) administered by the 10 in ovo or SC route to SPF chickens against a velogenic genotype V (Chimalhuacan) NDV challenge performed at 28 days of age.

The characteristics of the vHVT306 recombinant vaccine candidate tested in this study are described in Table 38 below. 15 The single HVT-IBD vector vaccine vHVT13 was used as a control.

TABLE 38

Characteristics of the recombinant vaccine candidate used in this study					
Name	Parental virus	Promoter	F gene	Poly-A	Locus
vHVT306	vHVT13	SV40	Opt-VIId	Synthetic	SORF3-US2

On day -3, 40 SPF embryonated eggs aged around 18 days and 18 hours of incubation were randomly allocated into 2 groups of 20 eggs each. On D0, one group of 12 day-old SPF 30 chicks was added. The definition of groups is given in Table 39 below. The vaccination was performed on D-3 (in ovo route) or on D0 (SC route, in the back of the neck) and the 35 target dose of vHVT306 and vHVT13 was 2000 PFU/bird. For the in ovo route, hatchability, viability (until D28) and growth of the birds (between hatching and D28) were monitored.

On D28, 10 birds per group were challenged with virulent ND Chimalhuacan strain. The challenge was performed by the intramuscular (IM) route using 10^5 Egg Infectious Dose 40 (EID50) diluted in 0.2 ml of physiological sterile water. Birds were monitored until 14 days post-challenge. Specific 45 clinical signs and mortality were recorded. Any bird that showed specific symptoms for more than 2 days or was noted sick on D42 was taken into account for calculation of morbidity. Five and seven days post-challenge (i.e. on D33 and D35), oropharyngeal swab was taken from each surviving bird. All the swabs were analyzed by specific NDV qRT-PCR.

TABLE 39

Results of ND protection induced by vHVT306 MDV vectored candidate expressing both NDV F and IBDV VP2 genes administered by the SC or in ovo route into SPF chicks				
Group	Vaccine/route	Hatchability/viability (%)	Protection against mortality/morbidity	% birds shedding at 5 dpi/7 dpi (mean log ₁₀ titer*)
G1	vHVT13/in ovo	100%/100%	0%/0%	(not tested)
G2	vHVT306/in ovo	100%/100%	100%/100%	0% (2.7)/0% (2.7)
G3	vHVT306/SC	—	100%/100%	20% (3.2)/10% (2.9)

*The threshold titer of the real time RT PCR was set at 2.7 log₁₀ equivalent EID50

Full hatchability was recorded after in ovo vaccination in groups 1 and 2 and all hatched birds survived up to D28. No difference in body weights was detected between the two

groups at both D0 and D28 confirming the perfect safety of vHVT306 when administered in ovo. Results of protection are summarized in Table 39. All vHVT13-vaccinated control birds died by 4 days after ND challenge. Full clinical ND protection was induced by vHVT306 administered by both routes. Furthermore, no shedding was detected after in ovo administration whereas only a few birds shed detectable amount of challenge virus after SC administration.

In conclusion, this study demonstrates that the double HVT-ND+IBD vHVT306 induce excellent level of ND protection by SC or in ovo administration routes in a very severe heterologous NDV challenge model.

Example 25

Efficacy of Double HVT-ND+IBD (vHVT302, vHVT303 and vHVT304) Recombinant Vaccines, Against Challenge with a Classical IBDV Isolate on D15 in SPF Chickens

The aim of the study was to assess the early IBD efficacy of double HVT recombinants vHVT302, vHVT303 and vHVT304 recombinant constructs against a virulent infectious bursal disease virus (IBDV) challenge (Faragher 52/70 strain) performed at 15 days of age in SPF chickens.

The characteristics of the 3 double HVT-ND+IBD recombinant vaccine candidates tested in this study are described in the Table 40 below.

TABLE 40

Characteristics of the expression cassettes of double HVT recombinants					
Name	Parental virus	Promoter	F gene	Poly-A	Locus
vHVT302	vHVT13	US10	Opt-VII ^d	US10	US10
vHVT303	vHVT13	US10	Opt-V (CA02)	US10	US10
vHVT304	vHVT13	SV40	Opt-VII ^d	Synthetic	IG2

On D0, 40 one-day-old SPF chickens were randomly allocated into 4 groups of 10 birds including one control group (G1) that was vaccinated with vSB1-004, a SB-1 vector expressing NDV F gene. Five other SPF birds were kept unvaccinated and unchallenged for bursal/body weights evaluation. The birds were injected by subcutaneous injection in the neck at D0 with 0.2 mL of recombinant vaccines containing a target dose of 2000 pfu as described in the Table 41 below. On D15, blood sample was collected from all birds per group (10 birds per group except for groups 1 and 3 in which 1 bird died before blood sampling) for serological testing with the Kit ProFLOK® plus IBD (Synbiotics Corp). On D15, birds from all 4 groups were challenged by the eye drop (0.05 mL per bird) with 2.5 log 10 EID50.

TABLE 41

Study design and results of IBD efficacy					
Group	Vaccine at day-old	ELISA IBD+ titer (log10)	Number Dead/Sick (total) ¹	% protection ²	Mean bursal/body weight ratio ⁴
G1	vSB1-004	0.25	1/9 (9)	0%	0.0014
G2	vHVT302	2.6	0/1 (10)	80%	0.0043

TABLE 41-continued

Study design and results of IBD efficacy					
Group	Vaccine at day-old	ELISA IBD+ titer (log10)	Number Dead/Sick (total) ¹	% protection ²	Mean bursal/body weight ratio ⁴
G3	vHVT303	3.0	0/0 (9)	100%	0.0053
G4	vHVT304	2.4	0/0 (10)	80%	0.0034

¹Birds sick for more than 2 days or still sick on D 25 were considered as sick. The number in brackets is the total number of birds in the group that were challenged.

²Protection against clinical signs and severe bursal lesion (bursal score <3)

⁴The bursal/body weight ratio of the unvaccinated/unchallenged group was 0.0043.

Each group was monitored before and after challenge. IBDV clinical signs were recorded for 11 days after challenge (from D15 to D25). At the end of the post-challenge observation period (D25), all the surviving birds were euthanized and necropsied. Body and bursal weights were recorded. Each bursa of Fabricius (BF) was weighted then stored in individual recipients containing 4% formaldehyde for histology. Histological lesions of the bursa were scored according to the scale presented in Table 42.

TABLE 42

Scoring scale of histological lesions of the bursa of Fabricius*	
Score	Histology observation/lesions
0	No lesion, normal bursa
1	1% to 25% of the follicles show lymphoid depletion (i.e. less than 50% of depletion in 1 affected follicle), influx of heterophils in lesions
2	26% to 50% of the follicles show nearly complete lymphoid depletion (i.e. more than 75% of depletion in 1 affected follicle), affected follicles show necrosis and severe influx of heterophils may be detected
3	51% to 75% of the follicles show lymphoid depletion; affected follicles show necrosis lesions and a severe influx of heterophils is detected
4	76% to 100% of the follicles show nearly complete lymphoid depletion; hyperplasia and cyst structures are detected; affected follicles show necrosis and severe influx of heterophils is detected
5	100% of the follicles show nearly complete lymphoid depletion; complete loss of follicular structure, thickened and folded epithelium, fibrosis of bursal tissue

*sourced from Monograph No. 01/2008: 0587 of EU Pharmacopoeia "Avian Infectious Bursal Disease vaccine (live)"

A bird was considered as affected if it died and/or showed notable sign of disease and/or severe lesions of the bursa of Fabricius (i.e., histology score 3).

The mean ELISA IBD+ antibody titer expressed in log 10 before challenge is shown in Table 41. Significant titers were detected in all vaccinated groups that were significantly higher than that of the control group G1. The serology titer was slightly higher in G3 (vHVT303).

Severe clinical signs were observed after challenge in all 9 birds of the control group G1, which lead to the death of 1 bird. Only one vaccinated bird in G2 (vHVT302) showed clinical signs after challenge. Percentages of protection against severe bursal lesions are shown in Table 41 above. Significant IBD protection was observed in all vaccinated groups, a full protection being observed in G3 (vHVT303). The mean bursal/body weight ratios are also shown in Table 41. Ratios in all vaccinated groups were higher than those of the challenged control group G1 and not significantly different from the unvaccinated and unchallenged control group.

In conclusion, these data indicate that the three double HVT-IBD+ND tested in this study induced IBD antibodies and early IBD protection in a severe IBDV challenge model.

Example 26

Efficacy of Five Different HVT-ND Vaccine Candidates Against Challenges with Velogenic NDV ZJ1 (Genotype VII^d) Isolate at 14 Days of Age in SPF Chickens

The aim of the study was to assess the efficacy of 5 single HVT recombinant constructs (vHVT39, vHVT110, vHVT111, vHVT112 and vHVT113) expressing the NDV F gene against Newcastle disease challenge with velogenic NDV ZJ1 (genotype VII^d) isolate performed at 14 days of age in SPF chickens.

The characteristics of these 5 vaccine candidates are described in Table 43 below.

TABLE 43

Characteristics of the HVT-ND recombinant viruses used in the challenge study					
Name	Parental virus	Promoter	F gene*	Poly-A	Locus
vHVT039	HVT	MDV gB	Wtnm-Texas	SV40	IG1
vHVT110	HVT	MCMV IE	Wt-VII ^d	SV40	IG1
vHVT111	HVT	SV40	Wt-VII ^d	SV40	IG1
vHVT112	HVT	MCMV IE	Wt-YZCQ	SV40	IG1
vHVT113	HVT	MCMV IE	Wt-Texas	SV40	IG1

*Wt means that the wild type velogenic F gene sequence was used but the cleavage site was modified to that of a lentogenic virus. Wtnm means that the cleavage site of the wild type sequence was not modified. The Texas velogenic strain belongs to genotype IV and YZCQ to the genotype VII^d.

On D0, 72 one-day-old SPF chickens were randomly allocated into 5 groups of 12 birds (vaccinated) and 1 group of 12 birds (non-vaccinated controls). The birds were injected by subcutaneous injection in the neck at D0 with 0.2 mL of recombinant vaccines containing a target dose of 6000 pfu as described in Table 44 below. The birds were challenged by the intramuscular route on D14 with 5 log 10 EID50 of NDV ZJ1/2000 (genotype VII^d) velogenic strain.

TABLE 44

Results of ND efficacy			
% clinical protection			
Group	Vaccine at day-old (D 0)	Protection against mortality/morbidity	Mean shedding titer (log10) at 2/4 dpi
G1	—	0%/0%	3.5/— (all dead)
G2	vHVT039	25%/8%	2.5/4.8
G3	vHVT110	100%/83%	1.8/2.0
G4	vHVT111	100%/67%	1.8/2.8
G5	vHVT112	75%/42%	1.7/3.4
G6	vHVT113	83%/25%	1.4/3.3

Each group was monitored before and after challenge. NDV clinical signs and mortality were recorded after challenge. Oropharyngeal swabs were taken at 2 and 4 days post-infection (dpi) for evaluation of viral load by real time RT-PCR using the method described by Wise et al. (2004; Development of a Real-Time Reverse-Transcription PCR for Detection of Newcastle Disease Virus RNA in Clinical Samples. J Clin Microbiol 42, 329-338).

Percentages of protection against mortality and morbidity are reported in the table 44 above. Full susceptibility was observed in the non-vaccinated challenged control group G1 thus validating the high severity of the challenge. Vaccines induced variable levels of protection against mortality (25-100%) or against morbidity (8%-83%). The best protection level was induced by vHVT110 whereas the lowest one was induced by vHVT039, the other candidates giving intermediate results. Results of oropharyngeal shedding at 2 and 4 dpi are also shown in Table 44 above and are in line with those of clinical protection. These vaccine candidates differ in their promoter and F gene sequence. These results show that both of these parameters are important for the design of optimal HVT-ND vaccine candidate.

In conclusion, the results of this study showed the importance of promoter and F gene sequence in the ND efficacy induced by HVT-vectorized ND vaccine candidates.

Example 27

Evaluation of the Newcastle Disease Efficacy Induced by Double SB1 Constructs Expressing IBDV VP2 and NDV F

The aim of the study is to assess the efficacy of double SB1 constructs expressing IBDV VP2 and NDV F against Newcastle disease challenge.

On D0, one-day-old SPF chickens are randomly allocated into several groups of 10-20 birds, including vaccinated and non-vaccinated groups. The birds of the vaccinated groups are injected by subcutaneous injection in the neck at D0 with 0.2 mL containing a target dose of 1000 to 5000 pfu of recombinant vaccines. Alternatively, the same dose in 0.05 mL may be administered in ovo 2 or 3 days before hatch. The birds (at least one vaccinated and one non vaccinated group) are challenged by the intramuscular route at different time after vaccination: for instance, D14, D28 or D42 with about 4.0 log 10 EID50 (0.1 mL) of a velogenic NDV strain such as Texas GB (genotype II), ZJ1 (genotype VII^d), Chimalhuacan (genotype V) strain.

Each group is monitored clinically before and after challenge. NDV clinical signs (morbidity) and mortality are recorded after challenge. Percentages of clinical protection in all groups are calculated. At least 90% of non-vaccinated challenged SPF birds should die or be severely sick after challenge to validate the severity of challenge. Oropharyngeal and cloacal swabs can be samples at different times after challenge such as 3, 5, 7 and 9 days post-challenge and the viral load can be estimated by real-time RT-PCR. The best candidates will be those who induced the highest level of clinical protection and the lowest level of viral load in the swabs. A similar study can be performed in broilers containing NDV maternal antibodies; however, these maternal antibodies may potentially protect the non-vaccinated birds if the challenge is performed early. The double SB1 construct may also be tested in combination with other Marek's disease vaccine or vector vaccines.

Example 28

Evaluation of the Infectious Bursal Disease Efficacy Induced by Double SB1 Constructs Expressing IBDV VP2 and NDV F

The aim of the study is to assess the IBD efficacy of double SB1 expressing both the IBDV VP2 and the NDV F.

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One-day-old SPF chickens are randomly allocated into several groups of 10 to 20 birds including vaccinated and non-vaccinated controls. Non-vaccinated controls will be separated into 2 subgroups including challenged and unchallenged birds. The birds of vaccinated groups are injected by subcutaneous injection in the neck at D0 with 0.2 mL of vaccines containing each a target dose of 1000 to 5000 pfu. Alternatively, the same dose in 0.05 mL may be administered in ovo 2 or 3 days before hatch. At different times after vaccination such as 14, 21, 28 or 42 days post-vaccination, all birds from vaccinated groups and the challenged controls are challenged by the eye drop (0.03 mL containing 2 to 4 log 10 EID50 per bird) of a virulent IBDV (such as the Faragher or the US standard strain), a very virulent IBDV such as the 91-168 isolate or a variant IBDV isolate such as the US Delaware variant E isolate. Each group is clinically monitored before and after challenge. Birds can be necropsied 4 or 5 days post-challenge for bursal gross lesions evaluation. They can also be necropsied 10 to 11 days post-challenge. Gross and/or histological lesions can be evaluated. Furthermore, birds and bursa are weighed the bursal/body weight ratios (bursa weight/body weight ratio $\times 100$) are calculated compared to those of the non-vaccinated unchallenged group. Control SPF challenged birds must show clinical signs and/or have significant gross and/or histological lesions, and/or should have a bursal/body weight ratio significantly lower than the unvaccinated unchallenged control birds to validate the severity of challenge. The efficacy of the vaccine is evaluated by comparing these parameters with unvaccinated/challenged and unvaccinated/unchallenged groups. Such study may be performed in broiler chickens containing IBDV maternal antibodies; however, these maternal antibodies may potentially protect the non-vaccinated birds if the challenge is performed early. The double SB1 construct may also be tested in combination with other Marek's disease vaccine or vector vaccines.

Example 29

Evaluation of the Marek's Disease Efficacy Induced by Double SB1 Constructs Expressing IBDV VP2 and NDV F

The aim of the study is to evaluate Marek's disease efficacy induced by the SB1 vectors expressing both IBDV VP2 and NDVF.

One-day-old SPF chickens are randomly allocated into several groups of 20 to 50 birds including vaccinated and

56

non-vaccinated controls. Non-vaccinated controls may be separated into 2 subgroups including challenged and unchallenged birds. The birds of vaccinated groups are injected by subcutaneous injection in the neck at D0 with 0.2 mL of vaccines containing each a target dose of 1000 to 5000 pfu. Alternatively, the same dose in 0.05 mL may be administered in ovo 2 or 3 days before hatch. At different times after vaccination such as 3 to 10 days post-vaccination, all birds from vaccinated groups and the challenged controls are challenged by the intraperitoneal route with 0.2 mL of a Marek's disease virus (MDV) strain. MDV strain may be of several pathotypes such as virulent MDV (vMDV) including the JM or GA22 isolate, very virulent MDV (vvMDV) such as the RB-1B or Md5 isolate, very virulent plus (vv+MDV) such as the T-King or 648A isolate. MDV challenge strain inoculum are prepared by infecting chickens, harvesting and freezing their blood cells into liquid nitrogen in presence of a cryopreservative such as DMSO. The chicken infectious dose 50 (CID50) is established for each challenge batch before performing vaccination/challenge studies. Each group is clinically monitored before and after challenge. Birds are necropsied after at least 7 weeks post-vaccination and the presence Marek's disease gross lesions is checked in each bird. Lesions might include, but not be limited to, the following: liver, heart, spleen, gonads, kidneys, nerve and muscle lesions. Such study may be performed in broiler chickens containing MDV maternal antibodies. The double SB1 construct may also be tested in combination with other Marek's disease vaccine (for instance HVT and or CVI988 Rispens strains) or MD vector vaccines. MD challenge may also be performed by contact between vaccinated birds and MDV infected non-vaccinated SPF chicks.

Having thus described in detail preferred embodiments of the present invention, it is to be understood that the invention defined by the above examples is not to be limited to particular details set forth in the above description as many apparent variations thereof are possible without departing from the spirit or scope of the present invention.

All documents cited or referenced herein ("herein cited documents"), and all documents cited or referenced in herein cited documents, together with any manufacturer's instructions, descriptions, product specifications, and product sheets for any products mentioned herein or in any document incorporated by reference herein, are hereby incorporated herein by reference, and may be employed in the practice of the invention.

SEQUENCE LISTING

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Asp	Gly	Arg	Pro	Leu	Ala	Ala	Ala	Gly	Ile	Val	Val	Thr	Gly	Asp	Lys
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Ala	Val	Asn	Val	Tyr	Thr	Ser	Ser	Gln	Thr	Gly	Ser	Ile	Ile	Val	Lys
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Leu	Leu	Pro	Asn	Met	Pro	Arg	Asp	Lys	Glu	Ala	Cys	Ala	Lys	Ala	Pro
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Leu	Glu	Ala	Tyr	Asn	Arg	Thr	Leu	Thr	Leu	Leu	Thr	Pro	Leu	Gly
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Lys Gln Gly Arg Leu Ile Gly Ala Val Ile Gly Ser Val Ala Leu Gly
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 130 135 140

Asn Gln Asn Ala Ala Asn Ile Leu Arg Leu Lys Glu Ser Ile Ala Ala
 145 150 155 160

Thr Asn Glu Ala Val His Glu Val Thr Asp Gly Leu Ser Gln Leu Ser
 165 170 175

Val Ala Val Gly Lys Met Gln Gln Phe Val Asn Asp Gln Phe Asn Asn
 180 185 190

Thr Ala Arg Glu Leu Asp Cys Ile Lys Ile Thr Gln Gln Val Gly Val
 195 200 205

Glu Leu Asn Leu Tyr Leu Thr Glu Leu Thr Thr Val Phe Gly Pro Gln
 210 215 220

Ile Thr Ser Pro Ala Leu Thr Gln Leu Thr Ile Gln Ala Leu Tyr Asn
 225 230 235 240

Leu Ala Gly Gly Asn Met Asp Tyr Leu Leu Thr Lys Leu Gly Ile Gly
 245 250 255

Asn Asn Gln Leu Ser Ser Leu Ile Gly Ser Gly Leu Ile Thr Gly Tyr
 260 265 270

Pro Ile Leu Tyr Asp Ser Gln Thr Gln Leu Leu Gly Ile Gln Val Asn
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Tyr Cys Ile Glu Ser Asp Leu Asp Leu Tyr Cys Thr Arg Ile Val Thr
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Phe Pro Met Ser Pro Gly Ile Tyr Ser Cys Leu Ser Gly Asn Thr Ser
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Ala Cys Met Tyr Ser Lys Thr Glu Gly Ala Leu Thr Thr Pro Tyr Met
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Ala Leu Lys Gly Ser Val Ile Ala Asn Cys Lys Ile Thr Thr Cys Arg
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Cys Thr Asp Pro Pro Gly Ile Ile Ser Gln Asn Tyr Gly Glu Ala Val
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Ser Leu Ile Asp Arg His Ser Cys Asn Val Leu Ser Leu Asp Gly Ile
 420 425 430

Thr Leu Arg Leu Ser Gly Glu Phe Asp Ala Thr Tyr Gln Lys Asn Ile
 435 440 445

Ser Ile Leu Asp Ser Gln Val Ile Val Thr Gly Asn Leu Asp Ile Ser
 450 455 460

Thr Glu Leu Gly Asn Val Asn Asn Ser Ile Ser Asn Ala Leu Asp Arg
 465 470 475 480

Leu Ala Glu Ser Asn Ser Lys Leu Glu Lys Val Asn Val Arg Leu Thr
 485 490 495

Ser Thr Ser Ala Leu Ile Thr Tyr Ile Val Leu Thr Val Ile Ser Leu
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Val Phe Gly Ala Leu Ser Leu Val Leu Ala Cys Tyr Leu Met Tyr Lys

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35         40          45

Ala Val Asn Val Tyr Thr Ser Ser Gln Thr Gly Ser Ile Ile Val Lys
50         55          60

Leu Leu Pro Asn Met Pro Arg Asp Lys Glu Ala Cys Ala Lys Ala Pro
65         70          75          80

Leu Glu Ala Tyr Asn Arg Thr Leu Thr Thr Leu Leu Thr Pro Leu Gly
85         90          95

Asp Ser Ile Arg Lys Ile Gln Gly Ser Val Ser Thr Ser Gly Gly Gly
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Lys Gln Gly Arg Leu Ile Gly Ala Val Ile Gly Ser Val Ala Leu Gly
115        120         125

Val Ala Thr Ala Ala Gln Ile Thr Ala Ala Ala Leu Ile Gln Ala
130        135         140

Asn Gln Asn Ala Ala Asn Ile Leu Arg Leu Lys Glu Ser Ile Ala Ala
145        150         155         160

Thr Asn Glu Ala Val His Glu Val Thr Asp Gly Leu Ser Gln Leu Ser
165        170         175

Val Ala Val Gly Lys Met Gln Gln Phe Val Asn Asp Gln Phe Asn Asn
180        185         190

Thr Ala Arg Glu Leu Asp Cys Ile Lys Ile Thr Gln Gln Val Gly Val
195        200         205

Glu Leu Asn Leu Tyr Leu Thr Glu Leu Thr Thr Val Phe Gly Pro Gln
210        215         220

Ile Thr Ser Pro Ala Leu Thr Gln Leu Thr Ile Gln Ala Leu Tyr Asn
225        230         235         240

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245        250         255

Asn Asn Gln Leu Ser Ser Leu Ile Gly Ser Gly Leu Ile Thr Gly Tyr
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Leu Pro Ser Val Gly Asn Leu Asn Asn Met Arg Ala Thr Tyr Leu Glu
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Lys Val Val Thr Gln Val Gly Ser Val Ile Glu Glu Leu Asp Thr Ser
325        330         335

Tyr Cys Ile Glu Ser Asp Leu Asp Leu Tyr Cys Thr Arg Ile Val Thr
340        345         350

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Ser Thr Ser Ala Leu Ile Thr Tyr Ile Val Leu Thr Val Ile Ser Leu
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Val Phe Gly Ala Leu Ser Leu Val Leu Ala Cys Tyr Leu Met Tyr Lys
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<212> TYPE: PRT
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<220> FEATURE:
<223> OTHER INFORMATION: NDV-F protein sequence from codon-optimized
CA02 gene

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20          25          30

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Asp Gly Arg Pro Leu Ala Ala Ala Gly Ile Val Val Thr Gly Asp Lys
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Ala Val Asn Ile Tyr Thr Ser Ser Gln Thr Gly Ser Ile Ile Ile Lys
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Leu Leu Pro Asn Met Pro Lys Asp Lys Glu Ala Cys Ala Lys Ala Pro
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Leu Glu Ala Tyr Asn Arg Thr Leu Thr Thr Leu Leu Thr Pro Leu Gly
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Asp Ser Ile Arg Arg Ile Gln Gly Ser Ala Thr Thr Ser Gly Gly Gly
100         105         110

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Lys Gln Gly Arg Leu Val Gly Ala Ile Ile Gly Ser Val Ala Leu Gly
115         120         125

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Val Ala Thr Ala Ala Gln Ile Thr Ala Ala Ala Leu Ile Gln Ala
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Asn Gln Asn Ala Ala Asn Ile Leu Arg Leu Lys Glu Ser Ile Ala Ala
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Thr Asn Asp Ala Val His Glu Val Thr Asn Gly Leu Ser Gln Leu Ala
165         170         175

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Val Ala Val Gly Lys Met Gln Gln Phe Val Asn Asn Gln Phe Asn Asn
180         185         190

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Thr Ala Arg Glu Leu Asp Cys Ile Lys Ile Ala Gln Gln Val Gly Val
195         200         205

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Glu Leu Asn Leu Tyr Leu Thr Glu Leu Thr Thr Val Phe Gly Pro Gln
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Ile Thr Ser Pro Ala Leu Thr Gln Leu Thr Ile Gln Ala Leu Tyr Asn
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Leu Ala Gly Gly Asn Met Asp Tyr Leu Leu Thr Lys Leu Gly Val Gly
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Asn Asn Gln Leu Ser Ser Leu Ile Gly Ser Gly Leu Ile Thr Gly Asn
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Leu Pro Ser Val Gly Ser Leu Asn Asn Met Arg Ala Thr Tyr Leu Glu
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305 310 315 320

Lys Val Val Thr Gln Val Gly Ser Val Ile Glu Glu Leu Asp Thr Ser
325 330 335

Tyr Cys Ile Glu Ser Asp Ile Asp Leu Tyr Cys Thr Arg Val Val Thr
340 345 350

Phe Pro Met Ser Pro Gly Ile Tyr Ser Cys Leu Ser Gly Asn Thr Ser
355 360 365

Ala Cys Met Tyr Ser Lys Thr Glu Gly Ala Leu Thr Thr Pro Tyr Met
370 375 380

Ala Leu Lys Gly Ser Val Ile Ala Asn Cys Lys Met Thr Thr Cys Arg
385 390 395 400

Cys Ala Asp Pro Pro Gly Ile Ile Ser Gln Asn Tyr Gly Glu Ala Val
405 410 415

Ser Leu Ile Asp Lys His Ser Cys Ser Val Leu Ser Leu Asp Gly Ile
420 425 430

Thr Leu Arg Leu Ser Gly Glu Phe Asp Ala Thr Tyr Gln Lys Asn Ile
435 440 445

Ser Ile Leu Asp Ser Gln Val Ile Val Thr Gly Asn Leu Asp Ile Ser
450 455 460

Thr Glu Leu Gly Asn Val Asn Asn Ser Ile Ser Ser Thr Leu Asp Lys
465 470 475 480

Leu Ala Glu Ser Asn Asn Lys Leu Asn Lys Val Asn Val Asn Leu Thr
485 490 495

Ser Thr Ser Ala Leu Ile Thr Tyr Ile Val Leu Ala Ile Val Ser Leu
500 505 510

Ala Phe Gly Val Ile Ser Leu Val Leu Ala Cys Tyr Leu Met Tyr Lys
515 520 525

Gln Arg Ala Gln Gln Lys Thr Leu Leu Trp Leu Gly Asn Asn Thr Leu
530 535 540

Asp Gln Met Arg Ala Thr Thr Arg Thr
545 550

<210> SEQ_ID NO 7
<211> LENGTH: 1362
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: IBDV DNA sequence encoding VP2

<400> SEQUENCE: 7

atgacaaaacc	tgcaagatca	aacctaacag	attgttccgt	tcatacggag	ccttctgtat	60
ccaaacaaccg	gaccggcgctc	cattccggac	gacaccctgg	agaaggcacac	tctcaggta	120
gagacctcgta	octacaattt	gactgtgggg	gacacaggg	cagggcta	tgtcttttc	180
cctggattcc	ctggctcaat	tgtgggtgt	cactacacac	tgcagagcaa	tgggaactac	240
aagttcgatc	agatgtccct	gactgcccag	aacctaccgg	ccagctacaa	ctactgcaga	300
ctagttagtc	ggagtctcac	agttaggtca	agcacactcc	ctggtaggcgt	ttatgcacta	360
aacggcacca	taaacgccgt	gaccttccaa	ggaagcctga	gtgaactgac	agatgttagc	420

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tacaatgggt	tgatgtctgc	aacagccaac	atcaacgaca	aaattggaa	tgtcctggta	480
ggggaaagggg	tcactgtcct	cagecttaccc	acatcatatg	atcttgggtt	tgtgaggctt	540
ggtgacccca	ttcccgctat	agggcttgcac	ccaaaaatgg	tagctacatg	cgacagcagt	600
gacaggccca	gagtctacac	cataactgca	gccgatgatt	accaattctc	atcacagttac	660
caaccaggtt	gggttaacaat	cacactgttc	tcagccaaaca	ttgtatgtat	cacaagctc	720
agcattgggg	gagagcttgt	gtttcaaaca	agcgtccaag	gccttgtact	gggcgccacc	780
atctacctta	taggctttga	tgggactgctg	gtaatcacca	gagctgttagc	cgcagataat	840
gggctgacgg	cgggcaccga	caatctttag	ccattcaatc	ttgtcattcc	aaccaatgag	900
ataaccacgc	caatcacatc	catcaaactg	gagatagtga	cctccaaaag	tggtggtcag	960
gcaggggatc	agatgtcatg	gtcggcaagt	gggagcctag	cagtgcacat	ccatgggtgc	1020
aactatccag	gggcctccg	tcccgtcaca	ctagtagcct	acgaaagagt	ggcaacagga	1080
tccgtcgat	cggtcgctgg	ggtgagtaac	ttcgagctga	ttccaaatcc	tgaactagca	1140
aagaacctgg	ttacagaata	cggccgattt	gaccaggag	ccatgaacta	cacaaaattt	1200
atactgagtg	agagggaccg	tcttggcatc	aagaccgtct	ggccaacaag	ggagtacact	1260
gattttcgtt	agtacttcat	ggaggtggcc	gacctaact	ctccccctgaa	gattgcagga	1320
gcatttggct	tcaaagacat	aatccggct	ataaggaggt	aa		1362

<210> SEQ ID NO 8

<211> LENGTH: 453

<212> TYPE: PRT

<213> ORGANISM: artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: IBDV VP2 protein

<400> SEQUENCE: 8

Met	Thr	Asn	Leu	Gln	Asp	Gln	Thr	Gln	Gln	Ile	Val	Pro	Phe	Ile	Arg
1							5		10				15		

Ser	Leu	Leu	Met	Pro	Thr	Thr	Gly	Pro	Ala	Ser	Ile	Pro	Asp	Asp	Thr
							20		25				30		

Leu	Glu	Lys	His	Thr	Leu	Arg	Ser	Glu	Thr	Ser	Thr	Tyr	Asn	Leu	Thr
							35		40			45			

Val	Gly	Asp	Thr	Gly	Ser	Gly	Leu	Ile	Val	Phe	Phe	Pro	Gly	Phe	Pro
							50		55			60			

Gly	Ser	Ile	Val	Gly	Ala	His	Tyr	Thr	Leu	Gln	Ser	Asn	Gly	Asn	Tyr
							65		70			75			80

Lys	Phe	Asp	Gln	Met	Leu	Leu	Thr	Ala	Gln	Asn	Leu	Pro	Ala	Ser	Tyr
							85		90			95			

Asn	Tyr	Cys	Arg	Leu	Val	Ser	Arg	Ser	Leu	Thr	Val	Arg	Ser	Ser	Thr
							100		105			110			

Leu	Pro	Gly	Gly	Val	Tyr	Ala	Leu	Asn	Gly	Thr	Ile	Asn	Ala	Val	Thr
							115		120			125			

Phe	Gln	Gly	Ser	Leu	Ser	Glu	Leu	Thr	Asp	Val	Ser	Tyr	Asn	Gly	Leu
							130		135			140			

Met	Ser	Ala	Thr	Ala	Asn	Ile	Asn	Asp	Lys	Ile	Gly	Asn	Val	Leu	Val
							145		150			155			160

Gly	Glu	Gly	Val	Thr	Val	Leu	Ser	Leu	Pro	Thr	Ser	Tyr	Asp	Leu	Gly
							165		170			175			

Tyr	Val	Arg	Leu	Gly	Asp	Pro	Ile	Pro	Ala	Ile	Gly	Leu	Asp	Pro	Lys
							180		185			190			

Met	Val	Ala	Thr	Cys	Asp	Ser	Ser	Arg	Pro	Arg	Val	Tyr	Thr	Ile	
-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	--

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195	200	205
Thr Ala Ala Asp Asp Tyr Gln Phe Ser Ser Gln Tyr Gln Pro Gly Gly		
210	215	220
Val Thr Ile Thr Leu Phe Ser Ala Asn Ile Asp Ala Ile Thr Ser Leu		
225	230	235
Ser Ile Gly Gly Glu Leu Val Phe Gln Thr Ser Val Gln Gly Leu Val		
245	250	255
Leu Gly Ala Thr Ile Tyr Leu Ile Gly Phe Asp Gly Thr Ala Val Ile		
260	265	270
Thr Arg Ala Val Ala Ala Asp Asn Gly Leu Thr Ala Gly Thr Asp Asn		
275	280	285
Leu Met Pro Phe Asn Leu Val Ile Pro Thr Asn Glu Ile Thr Gln Pro		
290	295	300
Ile Thr Ser Ile Lys Leu Glu Ile Val Thr Ser Lys Ser Gly Gly Gln		
305	310	315
Ala Gly Asp Gln Met Ser Trp Ser Ala Ser Gly Ser Leu Ala Val Thr		
325	330	335
Ile His Gly Gly Asn Tyr Pro Gly Ala Leu Arg Pro Val Thr Leu Val		
340	345	350
Ala Tyr Glu Arg Val Ala Thr Gly Ser Val Val Thr Val Ala Gly Val		
355	360	365
Ser Asn Phe Glu Leu Ile Pro Asn Pro Glu Leu Ala Lys Asn Leu Val		
370	375	380
Thr Glu Tyr Gly Arg Phe Asp Pro Gly Ala Met Asn Tyr Thr Lys Leu		
385	390	395
Ile Leu Ser Glu Arg Asp Arg Leu Gly Ile Lys Thr Val Trp Pro Thr		
405	410	415
Arg Glu Tyr Thr Asp Phe Arg Glu Tyr Phe Met Glu Val Ala Asp Leu		
420	425	430
Asn Ser Pro Leu Lys Ile Ala Gly Ala Phe Gly Phe Lys Asp Ile Ile		
435	440	445
Arg Ala Ile Arg Arg		
450		

<210> SEQ ID NO 9
<211> LENGTH: 368
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: SV40 promoter

<400> SEQUENCE: 9

```
gaattcgagc tcggatcagc ttggctgtgg aatgtgtgtc agttagggtg tggaaagtcc      60
ccaggctccc cagcaggcag aagtatgcaa agcatgcac tcaatttagtc agcaaccagg     120
tgtggaaagt ccccaggctc cccagcaggc agaagtatgc aaagcatgca tctcaatttag    180
tcagcaacca tagtcccgcc cctaactccg cccatcccg ccctaactcc gcccagtcc     240
gcccattctc cgccccatgg ctgactaatt ttttttattt atgcagaggc cgaggccgcc    300
tcggcctctg agctattcca gaagtagtga ggaggcttt ttggaggcct aggctttgc    360
aaaaagct                                         368
```

<210> SEQ ID NO 10
<211> LENGTH: 1391
<212> TYPE: DNA
<213> ORGANISM: artificial sequence

-continued

<220> FEATURE:

<223> OTHER INFORMATION: CMV-IE promoter

<400> SEQUENCE: 10

```

aactccgccc gtttatgac tagaaccaat agttttaat gccaaatgca ctgaaatccc      60
ctaatttgc aagccaaacg ccccttatgt gagtaatacg gggactttt acccaatttc      120
ccaagcggaa agccccctaa tacactcata tggcatatga atcagcacgg tcatgcactc      180
taatggcggc ccataggac ttccacata gggggcggtc accattccc agcataggg      240
tggtgactca atggcctta cccaaagtaca ttgggtcaat gggaggttaag ccaatgggt      300
tttccccatta ctggcaagca cactgagtca aatgggactt tccactgggt tttgeccaag      360
tacattgggt caatgggagg tgagccatg ggaaaaaccc attgctgcca agtacactga      420
ctcaataggg actttccaat gggttttcc attgtggca agcatataag gtcaatgtgg      480
gtgagtcaat agggacttcc cattgttattc tgcccaagtac ataaggtcaa tagggggtga      540
atcaacagga aagtcccatt ggagccaaatg acactgcgtc aataggactt ttccatttgg      600
tttgcocag tacataaggt caataggggta tgagtcaatg ggaaaaaccc attggagcca      660
agtacactga ctcaataggg actttccattt gggttttcc cagttacataa ggtcaatagg      720
gggtgagtca acagggaaatg cccattggag ccaagttacat tgagtcaata gggacttcc      780
aatgggtttt gcccagtaca taaggtaat gggaggttaag ccaatgggtt tttccattt      840
ctggcacgtt tactgagtca tttagggactt tccaaatgggtt tttgcccagt acataaggc      900
aataggggtt aatcaacagg aaagtcccat tggagccaaatg tacactgagt caataggac      960
tttccatttgg gtttgcaca gtacaaaagg tcaatagggtt gtgagtcaat gggttttcc      1020
cattattggc acgtacataa ggtcaatagg ggtgagtcat tgggttttc cagccattt      1080
aattaaaacg ccatgtactt tccaccattt gacgtcaatg ggctatttggaa actaatgca      1140
cgtgacccctt aaacggactt tccaccattt tgattatgg gaaagtaccc ttctcgagcc      1200
aatacacgtt aatgggaaatg gaaagggcag cccaaacgtt acaccgcccc ggtttcccc      1260
tgaaaaattcc atatggcac gcattctatt ggctgagctt cgttctacgt gggtaataaga      1320
ggcgccacca gcgtcggtac cggtcgactt ttccgtctgtt ccaccgttggaa acgcagagct      1380
cctcgctgca g                                              1391

```

<210> SEQ ID NO 11

<211> LENGTH: 218

<212> TYPE: DNA

<213> ORGANISM: artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: SV40 polyA signal

<400> SEQUENCE: 11

```

ggggatccag acatgataag atacattgtt gatgttggac aaaccacaac tagaatgcag      60
tgaaaaaaaaaat gctttatggat tggaaatggat gatgttggat ttatgtttca gggttcaggg      120
agctgcaata aacaagttaa caacaacaat tgcattgtt ttatgtttca gggttcaggg      180
gaggtgtggg aggtttttcc ggatcctcta gagtcgac                                              218

```

<210> SEQ ID NO 12

<211> LENGTH: 155

<212> TYPE: DNA

<213> ORGANISM: artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic polyA signal

-continued

<400> SEQUENCE: 12

```
aataaaaatat ctttattttc attacatctg tgggttgggtt ttttgtgtga atcgatagta      60
ctaacatacg ctctccatca aaacaaaacg aaacaaaaca aacttagcaa ataggctgtc      120
cccagtgc aa gtgcagggtgc cagaacattt ctctt                                155
```

<210> SEQ ID NO 13
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 13

```
cgaacaaact tcatcgctat gc                                         22
```

<210> SEQ ID NO 14
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 14

```
taactcaaat gcgaaagcggtt gc                                         22
```

<210> SEQ ID NO 15
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 15

```
actgacaaca ccctacatgg c                                         21
```

<210> SEQ ID NO 16
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: VIIoptF RP primer

<400> SEQUENCE: 16

```
gccagcacca ggctcaggg                                         19
```

<210> SEQ ID NO 17
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 17

```
agcttggctg tggatgt                                         18
```

<210> SEQ ID NO 18
<211> LENGTH: 4344
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Partial plasmid pHM103+Fopt DNA sequence

<400> SEQUENCE: 18

```
gagctcaggg tatgataactc agctgttatt gtggccgacc aggaggactc caatgcttag      60
```

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cattcataag aacgctagag atgctattta acgatgtgct gtcgtctaaa gaatttgtgc	120
attttagcattt taaatgtaaa accaatgacg cattcaaac gtcgtgcgt gcaatttctg	180
ggccagggtt tgcatattcc ataacagaaa tcgacacttg agaagaggat ctgactgttt	240
gggataaaagg tcgtttgggt ctgtcctagc gatataattt atatgacgat atacattaaa	300
catctgtgtg cagtagttttt gtatattaatc atgtcgatga aatgttatgt gttaatatcg	360
gacaatatacg ataacggca cgctgctattt gtaacgtgcg cccgcgcgt agtgcgtact	420
aataatgtgg atgatgtata cagttatata caaacggaaa tgatacgtaa taaattatgt	480
actcttattt atttataaaa acatacatgc agtgttgcata tgtcacataa ttagectcgc	540
ccgtctacgc tccactgaag ataatggctt cccgctgttc aaaaaaatca gcgtgcgtcg	600
ataagacttt ggtgcagtct ctgcgggtc gcaatttaga ttgccgcatt ggagggtatc	660
tggggatttt tgccaatgct ggagcgacga ctgtacgatt cgtcccatcg ggatctagca	720
gaccaatgtatgtt gttgacacac atcggccatg catgtacggg cggcttattt cgcgcgttt	780
ttattttgcg aggacaagat ggaagtgtat atggaaaccga caataatgtt agtttgcatt	840
tcttagggcg gaatctacat gatactttt ccaagcgggg tatgagccag agagatgtga	900
tggtcataaa gggtaaattt tttagatctg aaataacgca gttgcggaaa caacgatcgc	960
gattaaaaga aaaatcggtt ggttcaatta ggacatgcgat ggattctgtg cgcataaacc	1020
ataacccgcg cactgttggg cacttcggta actcaaattgc gaagcgttgc acgtctgcga	1080
taactacgcc tactatgcac attgttactc ctgcattttt aaaaatatc ctgttagtaat	1140
tttcacagca atgtcataac atcatctcgc taaagaatga cctggattt gagaagtaat	1200
gaatatttgc aaccaatgca ttgataaaac taacattaaa cgaattcgag ctccgtacag	1260
cttggctgtg gaatgtgtgt cagttagggt gtggaaagtc cccaggctcc ccageaggca	1320
gaagtgatgc aagcatgcat ctcaattagt cagcaaccag gtgtggaaag tccccaggct	1380
ccccagcagg cagaagtttgc caaaggcatgc atctcaattt gtcagcaacc atagccgc	1440
ccctaactcc gccccatcccg cccctaactc cgcggcttc cgcgcattct ccgcggccatg	1500
gctgactaat ttttttattt tatgcagagg cccggccgc ctcggctct gagctattcc	1560
agaagtagtg aggaggctt tttggaggcc taggtttttt caaaaagctg cggccgcac	1620
catgggcgcg aagcccagca caagaatccc aaaaaaaaaatgtatca cccgcattcat	1680
gctgatcctg ggctgcatca gacccacaag ctccctggat ggacgcccc tggccgcgtc	1740
cgccatcgtg gtgaccggcg acaaggccgtt gaacgtgtac accagcagcc agaccggcag	1800
catcatcgtg aagctgctgc ccaacatgcc cagagacaaa gaggcctgcg ccaaggcccc	1860
cctggaaagcc tacaacagaa ccctgaccac cctgctgacc cccctggcg acagcatcag	1920
aaagatcccg ggctccgtga gcacaagccgg cggaggaaag cagggcagac tgatcgccgc	1980
cgtgatcggc acgctggccc tgggagtgcc tacagctgcc cagattaccg ctgcagccgc	2040
cctgatccag gccaaaccaga acgccccaa catcctgaga ctgaaagaga gcattgcgc	2100
ccaacacgg gcccgtgcacg aagtgaccga cggccctgagc cagctgtccg tggccgtggg	2160
caagatgcag cagttcgtga acgaccagtt caacaacacc gccagagagc tggactgcatt	2220
caagatcacc cagcagggtgg gcgtggagctt gaacctgtac ctgaccggc tgaccacagt	2280
gttcggcccc cagatcacaa gcccggccctt gacacagctg accatccagg ccctgtacaa	2340
cctggctggc ggcaacatgg actatctgtt gacaaagctg ggaatcgccca acaaccagct	2400

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gtccagctg atcggaaagcg gcctgatcac cggctacccc atcctgtacg acagccagac	2460
acagctgctg ggcattccagg tgaacctgcc cagcgtgggc aacctgaaca acatgcgcgc	2520
cacctacctg gaaaccctga gcgtgtccac caccaagggc tacgcccagcg ccctggtgcc	2580
caaggtggtg acacaggtgg gcagcgtgat cgaggaactg gacaccagct actgcatcga	2640
gagcgacctg gacctgtact gcaccagaat cgtgacccat ccaatgagcc ccggcatcta	2700
cagctgcctg agcggcaaca ccagcgcctg catgtacagc aagacccaag ggcactgac	2760
aacaccctac atggccctga agggaaagct gatgcaccaac tgcaagatca ccacctgcag	2820
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tgcgcattcc tgtaacgtgc tgtccctgga cggcatcaca ctgagactga gcccgcagtt	2940
cgtatccacc taccagaaga acatcagcat cctggacagc caggtgatcg tgaccggcaa	3000
cctggacatc agcaccggagc tggcaacgtt gaataacagc atcagcaacg ccctggacag	3060
actggccgag agcaacagca agctggaaaa agtgaacgtg cgcctgacat ccacttcgc	3120
tctgatcacc tacatcgtgc tgaccgtat cagcctgggt ttcggccccc tgagcctgg	3180
gttggcctgc tacctgtatgt acaaggcagaa ggcccagcg aaaaccctgc tgtggctgg	3240
caacaacacc ctggaccaga tgagagccac caccagagcc ttagtgagccg ccgcggggat	3300
ccagacatga taagatacat ttagtgatgtt ggacaaacca caactagaat gcagtggaaa	3360
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aataaacaag ttaacaacaa caattgcatt gattttatgt ttcaggttca gggggaggtg	3480
tgggaggttt ttccggatcc tctagagtgc acaattatttt tatttaataa catatagccc	3540
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acgttgtct tataatgtat gatcgatgtt tcaccctaat gccacatggt acaggcttat	3960
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tatagcctgt agactatttt tggttttaatgtt gtgaacgggg cgtgtgaac gagactcggg	4260
ccgattgtaa gaacaagcaa atgcacttcc catttaacaa gaagtgttgc gagaataactc	4320
aaccttttg gatgtatccctt cgag	4344

<210> SEQ ID NO 19
 <211> LENGTH: 4085
 <212> TYPE: DNA
 <213> ORGANISM: artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Partial plasmid pSB1_44cds SV FCAopt sequence
 for vSB1-009

<400> SEQUENCE: 19

cttttgtcat gtcggagct ctgatcgat cttatcatta cgtctgcata gcaacgtctg 60

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gagacgtgac gtggaaagacc gggtttttag ttgtggccgc agggacgatt gccggcatca	120
cggtccgtt tggagacatt tctctcttag cggctttctt ttccgggtat acgggttag	180
ctattcacgt ggtcagagac gccagtcgtt ctctaataa cacgtgtac taccgtgcac	240
gtcggaaat tactgtgaac ggtgcataatc gcctcggtcg cgccgcgttc ccggccagca	300
cggacgcccga ggccgacgcgc gaagaagacg tatccaggta cgatacgctg ggggggaata	360
ttcctacgt aattctgagc ctcataegg tcatactcgat tccagecata gccagcttc	420
aaaagtacat gtcgaacgca actaaggcacc agtcaacatt gactgacacg ttacgcagta	480
tatgcggttt ctgggtgggt acaagtgtcg cgatattctt tccgtcgcc taccacgagg	540
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ccggcttcgg tttacttctc gggccgacat tgtttccgc gacagccgcg gttctgtgct	660
gtacacttg tataaatgtt cgcaacgcga atagcggaaat aaagcaattt gccccccgcg	720
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aatattccctt atcgccggagc gattaatttt tatatacatgt gtcatacgcg ttcttcgaa	840
ctgcgaataaa aactttcggt gctactaaag gggcttatcg tgggtttatcg cgctgtcgaa	900
aacatgaaag ggccgattta aagctaagtt ggcaggcag aggccactcc atatacgctc	960
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<210> SEQ ID NO 20
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 <213> ORGANISM: artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Partial plasmid phVT US2 SV-Fopt-SynPA for vHVT306

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cattatggat atatcttccg gttgtccat atcccgccct ggtaccgctc ggataccctg	180
cccgatggta tcgatgttgcgat cagtcgcgc aatggggacc aacaacgcgt gggccacac	240
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tagcagatct cgcaacctcc agggaggcta taataacgtt tttaaaggat ggattctca	540
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tcgtccaatt ttctaaatgg aaagaaaaca aggccggcaa gagtgttcca aacatttca	660
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89

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<210> SEQ ID NO 21
 <211> LENGTH: 5381
 <212> TYPE: DNA
 <213> ORGANISM: artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: plasmid pCD046+NDV-F wt for vHVT110

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<210> SEQ ID NO 22
<211> LENGTH: 4337
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Partial plasmid pHM103+NDV-F wt sequence for vHVT111

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<210> SEQ ID NO 26
 <211> LENGTH: 3707
 <212> TYPE: DNA
 <213> ORGANISM: artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Partial plasmid pHVT US20 cds F CA02 opt sequence for vHVT303

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<210> SEQ_ID NO 27
<211> LENGTH: 3946
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Partial plasmid HVT IG2 SVFopt syn tail sequence for vHVT304

<400> SEQUENCE: 27

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<210> SEQ ID NO 28
<211> LENGTH: 4654
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Partial plasmid pHVT US2 SV-FCA02 opt-synPA for vHVT307

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 <212> TYPE: DNA
 <213> ORGANISM: artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: partial plasmid pCD046+NDV-F VII YZCQ sequence
 for HVT112

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<210> SEQ ID NO 30
 <211> LENGTH: 5381
 <212> TYPE: DNA
 <213> ORGANISM: artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: partial plasmid pCD046+Texas NDV-F sequence for HVT113

<400> SEQUENCE: 30

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ttaataacat atagccaaa gacctctatg aacattttagt ttcccgtata ctcaacggcg	4620
cgtgtacaca cgcacatcttt tgcatagcg tgaagtttgc tggcagcagc aaaaatgcaga	4680
tatccaacaa tctggagaaa acttatcatc acagtggcag tggaaacata cccccccttat	4740
attcatggta taattatcgt ctacagcgcc caggatgtt gctgtgagaaa atggagatct	4800
gcagccctcc tttccatggc atgcccctt attgttcat aaacgcacaa tggctcaac	4860
gcccggatgtt ggcatacgatt ctgaagaacc cgttgcacat ccgaagaaga aggccgtcag	4920
gtctttggaa gactcgcacg ttggcttattt aatgtatgtat cgagatgtca ccctaatgcc	4980
acatggtaca ggcttacatgc ggtcatggcg atcggacttg taatggcaaa cgatggcaaa	5040
aggatcgacg acatgcacaa cattctgaac ccgttagatgt gttaacgcg acgaggatga	5100
atatcccatg ctcgcgtccca tagtatacgat tacaccgcgaa ataaggacgc gtccaaacatc	5160
gttataatgca cacaatgggc tacacgtgac taacacccccc gaatattatgt catatgtgag	5220

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ttccagtctg gctccatat agcctgtaga ctatttgg ttaagtgtg aacgaggcgc 5280
 tgtgaacgag actcgcccg attgtaaagaa caagcaaatg cactttccat ttaacaagaa 5340
 gtgttagagag aataactcaac ctcttggat gtatcctcgag 5381

 <210> SEQ ID NO 31
 <211> LENGTH: 4600
 <212> TYPE: DNA
 <213> ORGANISM: artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: partial plasmid pHM119 sequence for vHVT039

 <400> SEQUENCE: 31

 gagctcaggg tatgatactc agctgttatt gtggccgacc aggaggactc caatgcttag 60
 cattcataag aacgctagag atgctattta acgatgtgc gtcgtctaaa gaatttgc 120
 atttagcatt taaatgtaaa accaatgacg cattcaaac gtcgtgcgt gcaatttctg 180
 ggccagggta tgcatattcc ataacagaaa tgcacacttg agaagaggat ctgactgttt 240
 gggataaagg tcgtttgggt ctgtcctagc gatataattt atatgacgat atacattaaa 300
 catctgtgtg cagtagttttagt gtatattaatc atgtcgatgaa atatgtatgt gtataatcg 360
 gacaatatacgataa cgcgtctatt gtaacgtgcg cccgcgcgcgt agtgcgtgact 420
 aatagtgtgg atgatgtata cagtagttttagt caaaacggaaa tgatacgtaa taaattatgt 480
 actcttatttgcg atttataaaaa acatacatgc agtgttgcata tgcacataa ttagectcgc 540
 ccgtctacgc tccactgaag ataatggctt cccgctgttc aaaaaaatca gcgtgcgtcg 600
 ataagactttt ggtgcgtctt ctgcggggc gcaattttaga tttgcgcattt ggagggtatc 660
 tggggattttt tgccaatgtctt ggagcgcacgat ctgtacgattt cgtccatcg ggtcttagca 720
 gaccaatgtatgtt gttgacacac atcggccatg catgtacgat cggcttattt cgcgagttt 780
 ttatccatcgataggat ggaagtgtat atggaaacgat caataatgtt agtggatcatt 840
 tcttagggcg gaatctacat gatatctt ccaagcgggg tatgagccag agagatgtga 900
 tggtcataaa gggtaaattttt ttttagatgtt aaataacgat gttgccaaa caacgatcg 960
 gattaaaaga aaaatcgat ggttcaattt ggacatgtat ggttctgtt cgcataaacc 1020
 ataacccgcg cactgttggg cacttcggta actcaaatgc gaagcgttgc acgtctcgat 1080
 taactacgccc tactatgcac attgttactc ctgcacatcta aaaatatactc ctgttagtaat 1140
 ttccacacgc atgtcataac atcatctcgat taaaatgtat cctggatgtt gagaagttat 1200
 gaatatttgc aaccaatgca ttgataaaac taacattaaa cgaattccga tggatgtca 1260
 cgatagacat cgggtcgccc agccgtcgaa tacagcatta tatttttagt gttaaaatgt 1320
 agggctgtctt cctactttaa aggaggaaat ggctcgatcc atgtttccatc gcaatgtaaa 1380
 aacagattgg accgtcagta agttagggat gttttagatgtt ttttagacta tagataatgt 1440
 aactgcggcc catcgatgg cttggaaata tatcaaagaa ctgatccatc caacagctt 1500
 attttcttctt gtatattaaat gtggcgttattt gcacatctgtt cgtgcgcata gtttgcagat 1560
 caacagacat gggactatgtt tatggaaaaaa tggatataatc ataacatgtt aaaccgaaata 1620
 tccacttata atgattctgg ggtcagaatc aagcacttca gaaacgcata atatgactgc 1680
 aattattgtt acagatgtttt tttcgatccat ttttagacta tggccccgtt 1740
 tacggcagat caggtgcgag tagaacatgtt tccaaacgatc cacgcggccca tctgaccgtt 1800
 ccaatattctt tttgtccctgtt cattttatctt cacacaattt atgaacagca tcattaaatgt 1860

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catctcactg cggccgcaag atgggctcca gatcttctac caggatcccg gtacctaa	1920
tgctgtatcat ccgaaccgcg ctgacactga gctgtatccg tctgacaagc tctcttgatg	1980
gcaggectct tgccgtcgca gggatcgtag taacaggaga taaagcagtc aacatataca	2040
cctcatcccc gacagggtca atcatagtta agttactccc gaatatgcc aaggacaaag	2100
aggtgtgtgc aaaagcccc a ttggaggcat acaacaggac actgactact ttactcaccc	2160
cccttggtga ttctatccgc aggatacaag agtctgtgac tacttccgga ggaaggagac	2220
agagacgctt tataagggtcc attatcgca gtgttagctct tggggttcg acagctgac	2280
agataaacagc agcttcggcc ctgatacaag ccaaccagaa tgctgccaac atcctccggc	2340
ttaaagagag cattgctgca accaatgaag ctgtgcacga ggtcaactgac ggattatcac	2400
aacttagcgt ggcagttaggg aagatgcaac agtttgc当地 tgaccaggta aataatacag	2460
cgcaagaatt ggactgtata aaaattgcac agcaggtcgg tgtagaactc aacttgc当地	2520
taactgaatt gactacagta tttgggcccac aaatcacttc ccctgcctta actcagctga	2580
ctatccaaggc gctttacaat ctatgtggg gtaatatggg ttacttgc当地 actaagttag	2640
gtgttagggaa caaccaactc agctcattaa ttggtagcgg ctgtatcacc ggcaacccta	2700
ttctgtacga ctcacagact cagatctgg gtatacaggta aactttgc当地 tcagttggg	2760
acctgaataa tatgcgtgcc acctacctgg agaccttatac tgtaaggcaca accaaggat	2820
ttgcctcaggc acttgtccca aaagtggta cacaggtcgg ttccgtgata gaagaacttgc	2880
acacctata ctgtataggg accgacttgg atttatactg tacaagaata gtgacattcc	2940
ctatgtctcc tggatattat tcttgc当地 gcggtatatac atcggcttgc atgtattcaa	3000
agactgaagg cgcaacttact acgccccata tggctctcaa aggctcagtt attgccaatt	3060
gcaagctgac aacatgtaga tggcagatc ccccaggat catatgc当地 aattatggag	3120
aagctgtgtc cttatagat aggcactcat gcaacgttcc atccttagac gggataactc	3180
tgaggctcag tggggatattt gatgcaacct atcaaaaagaa tatctctata ctatgtctc	3240
aagttatagt gacaggcaat cttgatatac caactgagct tggggatgtc aacaactcaa	3300
taagtaatgc cttatagat ttagaggaaa gcaacagcaa actagacaaa gtcaatgtca	3360
aactgaccag cacatctgct ctcattaccc acatcggtt aactgtcata tctcttgc当地	3420
ttgggtgact tagcctggg ttatctatgtc acctgtatgc当地 caagcaaaa gcacaacaaa	3480
agaccttgc当地 atggcttggg aataataccctt ttgatcagat gagagccact acaaaaat	3540
gagcggccgc ggggatccag acatgataag atacattgtatc gagtttggac aaaccacaa	3600
tagaatgc当地 tgaaaaaaat gctttatgg tggatattgt gatgtatgg ctttattgt	3660
aaccattata agctgcaata aacaagttaa caacaacaat tgcatttccat ttatgtttca	3720
ggttcagggg gaggtgtggg aggttttgc当地 ggatccctata gagtc当地 aacaactcaa	3780
taataacata tagcccaaag acctctatgc当地 acatgtatggt tccctatatac tcaacggcc	3840
gtgtacacac gcatctctt gcatagcgat gaagtttgc当地 cggcagcaga aaatgc当地	3900
atccaaacata ctggagaaaaa cttatcatca cagttgc当地 ggaaacatatac cccctctata	3960
ttcatgtat aattatcgatc tacagcgatc aggatgtgg cgtgagaaaa tggagatctg	4020
cagccctctt ttccatggca tgccgttta ttgttccatata aacgc当地 aatgtcaac	4080
ccagatatgg gcatagatcc tgaagaaccc gttgacaatc cgaagaagaa ggcgtgc当地	4140
tctttggaaag actcgacgt tggatattata atgtatgtatc gagatgtc当地 cctaattgc当地	4200
catggtacag gcttatcgcc gtc当地 tggacttgc当地 aatggcaac gatggcaaa	4260

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ggatcgacga catgccaaac attctgaacc cgttagagatg ttaacgtatgc cgaggatgaa	4320
tatccccatgc tcgctgccat agtatcaagt acaccgcgaa taaggacgcg tccaaatcg	4380
ttatatgcac acaatggct acacgtgact aacaccccg aatattagtc atatgtgagt	4440
ttcagtcgg ctccatata gcctgttagac tatttgggt ttaagtgtga acgaggcgct	4500
gtgaacgaga ctcggccga ttgtaaaac aagcaaattgc actttccatt taacaagaag	4560
tgttagagaga atactcaacc tctttggatg tattctcgag	4600

<210> SEQ ID NO 32
<211> LENGTH: 1662
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: NDV Texas F gene (wild type non-modified)

<400> SEQUENCE: 32	
atgggctcca gatcttctac caggatcccg gtacctctaa tgctgtatcat ccgaaccgcg	60
ctgacactga gctgtatccg tctgacaagg tctcttgatg gcaggccctt tgccggctca	120
gggatcgtgg taacaggaga taaaggcgtc aacatataaca cctcatccca gacagggtca	180
atcatagttt agttactccc gaatatgccc aaggacaaag aggtgtgtgc aaaagcccca	240
ttggaggcat acaacaggac actgactact ttactcaccc cccttgggtga ttctatccgc	300
aggatacaag agtctgtgac tacttccgga ggaaggagac agagacgctt tatagggcc	360
attateggca gtgtagctt tggggttcg acagctgcac agataacaggc agcttcggcc	420
ctgatacaag ccaaccagaa tgctgccaac atcctccggc ttaaagagag cattgctgca	480
accaatagaag ctgtgcacga ggtcaactgac ggattatcac aacttagcgt ggcagtaggg	540
aagatgcaac agtttgcacaa tgaccaggc aataatacag cgcaagaatt ggactgtata	600
aaaattgcac agcagggtcg tggtagactc aacttgcatt taactgaatt gactacagta	660
tttggccac aaatacttc ccctgcctta actcagctga ctatccaagg gctttacaat	720
ctagctgggt gtaatatggaa ttacttgcgt actaaggtag gtgttagggaa caaccaactc	780
agctcattaa ttggtagcggt cttgatcacc ggcaacccta ttctgtacga ctcacagact	840
cagatcttgg gtatacaggta aaccttgcgt tcagttggaa acctgaataa tatgegtgcc	900
acctacccgg agacccatc tgtaaggcaca accaaggat ttgcctcagc acttgcctca	960
aaagtgggtga cacagggtcg ttccgtgata gaagaacttg acacccata ctgtatagg	1020
accgacttgg atttatactg tacaagaata gtgacattcc ctatgtctcc tggattttat	1080
tcttgcgtga gcgtaatac atcggcttgc atgtattcaa aactgtgagg cgcaacttact	1140
acgccatata tggctctcaa aggctcgtt attgccaattt gcaactgtac aacatgtaga	1200
tgtgcagatc ccccgaggat catatcgaa aattatggag aagctgtgtc cttaatagat	1260
aggcactcat gcaacgtctt atccttagac gggataactc tgaggctcag tggggat	1320
gatgcaacct atcaaaagaa tatctctata cttagattctc aagttatagt gacaggcaat	1380
cttgatataat caactgagct tgggaatgtc aacaactcaa taagtaatgc cctgtataa	1440
ttagaggaaa gcaacagcaa actagacaaa gtcaatgtca aactgaccag cacatctgt	1500
cttcattaccc acatcggttt aactgtcata tctcttggtt ttgggtgtact tagcctgtt	1560
ctagcatgct acctgtatgtaa caagcaaaa gcaacaacaa agacccgtt atggcttggg	1620
aataataccctt tgatcagat gagagccact acaaaaaatataat ga	1662

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<210> SEQ ID NO 33
<211> LENGTH: 553
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: NDV Texas F protein (wild type non-modified)

<400> SEQUENCE: 33

Met Gly Ser Arg Ser Ser Thr Arg Ile Pro Val Pro Leu Met Leu Ile
1           5          10          15

Ile Arg Thr Ala Leu Thr Leu Ser Cys Ile Arg Leu Thr Ser Ser Leu
20          25          30

Asp Gly Arg Pro Leu Ala Ala Gly Ile Val Val Thr Gly Asp Lys
35          40          45

Ala Val Asn Ile Tyr Thr Ser Ser Gln Thr Gly Ser Ile Ile Val Lys
50          55          60

Leu Leu Pro Asn Met Pro Lys Asp Lys Glu Val Cys Ala Lys Ala Pro
65          70          75          80

Leu Glu Ala Tyr Asn Arg Thr Leu Thr Thr Leu Leu Thr Pro Leu Gly
85          90          95

Asp Ser Ile Arg Arg Ile Gln Glu Ser Val Thr Thr Ser Gly Gly Arg
100         105         110

Arg Gln Arg Arg Phe Ile Gly Ala Ile Ile Gly Ser Val Ala Leu Gly
115         120         125

Val Ala Thr Ala Ala Gln Ile Thr Ala Ala Ser Ala Leu Ile Gln Ala
130         135         140

Asn Gln Asn Ala Ala Asn Ile Leu Arg Leu Lys Glu Ser Ile Ala Ala
145         150         155         160

Thr Asn Glu Ala Val His Glu Val Thr Asp Gly Leu Ser Gln Leu Ala
165         170         175

Val Ala Val Gly Lys Met Gln Gln Phe Val Asn Asp Gln Phe Asn Asn
180         185         190

Thr Ala Gln Glu Leu Asp Cys Ile Lys Ile Ala Gln Gln Val Gly Val
195         200         205

Glu Leu Asn Leu Tyr Leu Thr Glu Leu Thr Thr Val Phe Gly Pro Gln
210         215         220

Ile Thr Ser Pro Ala Leu Thr Gln Leu Thr Ile Gln Ala Leu Tyr Asn
225         230         235         240

Leu Ala Gly Gly Asn Met Asp Tyr Leu Leu Thr Lys Leu Gly Val Gly
245         250         255

Asn Asn Gln Leu Ser Ser Leu Ile Gly Ser Gly Leu Ile Thr Gly Asn
260         265         270

Pro Ile Leu Tyr Asp Ser Gln Thr Gln Ile Leu Gly Ile Gln Val Thr
275         280         285

Leu Pro Ser Val Gly Asn Leu Asn Asn Met Arg Ala Thr Tyr Leu Glu
290         295         300

Thr Leu Ser Val Ser Thr Thr Lys Gly Phe Ala Ser Ala Leu Val Pro
305         310         315         320

Lys Val Val Thr Gln Val Gly Ser Val Ile Glu Glu Leu Asp Thr Ser
325         330         335

Tyr Cys Ile Gly Thr Asp Leu Asp Leu Tyr Cys Thr Arg Ile Val Thr
340         345         350

Phe Pro Met Ser Pro Gly Ile Tyr Ser Cys Leu Ser Gly Asn Thr Ser
355         360         365

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Ala Cys Met Tyr Ser Lys Thr Glu Gly Ala Leu Thr Thr Pro Tyr Met
 370 375 380
 Ala Leu Lys Gly Ser Val Ile Ala Asn Cys Lys Leu Thr Thr Cys Arg
 385 390 395 400
 Cys Ala Asp Pro Pro Gly Ile Ile Ser Gln Asn Tyr Gly Glu Ala Val
 405 410 415
 Ser Leu Ile Asp Arg His Ser Cys Asn Val Leu Ser Leu Asp Gly Ile
 420 425 430
 Thr Leu Arg Leu Ser Gly Glu Phe Asp Ala Thr Tyr Gln Lys Asn Ile
 435 440 445
 Ser Ile Leu Asp Ser Gln Val Ile Val Thr Gly Asn Leu Asp Ile Ser
 450 455 460
 Thr Glu Leu Gly Asn Val Asn Asn Ser Ile Ser Asn Ala Leu Asn Lys
 465 470 475 480
 Leu Glu Glu Ser Asn Ser Lys Leu Asp Lys Val Asn Val Lys Leu Thr
 485 490 495
 Ser Thr Ser Ala Leu Ile Thr Tyr Ile Val Leu Thr Val Ile Ser Leu
 500 505 510
 Val Phe Gly Val Leu Ser Leu Val Leu Ala Cys Tyr Leu Met Tyr Lys
 515 520 525
 Gln Lys Ala Gln Gln Lys Thr Leu Leu Trp Leu Gly Asn Asn Thr Leu
 530 535 540
 Asp Gln Met Arg Ala Thr Thr Lys Ile
 545 550

<210> SEQ_ID NO 34
 <211> LENGTH: 1662
 <212> TYPE: DNA
 <213> ORGANISM: artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: NDV-F YZCQ wildtype DNA sequence

<400> SEQUENCE: 34

atgggctcca	gatcttctac	caggatcccg	gtacctctaa	tgctgtatcat	ccgaaccgcg	60
ctgacactga	gctgttatccg	tctgacaagc	tctcttgatg	gcaggcctct	tgcggctgca	120
gggatcgtgg	taacaggaga	taaaggcagtc	aacatataaca	cctcatccca	gacagggtca	180
attcatagtt	agttactccc	gaatatgcc	aaggacaaag	aggtgtgtgc	aaaagccccca	240
ttggaggcat	acaacaggac	actgactact	ttactcaccc	cccttggtga	ttcttatccgc	300
aggatatacg	agtctgtgac	tacttccgga	ggaggcaagc	aaggccgcct	gataggtgcc	360
attatcggca	gtgttagctct	tggggttgcg	acagctgcac	agataaacagc	agcttcggcc	420
ctgatacaag	ccaaaccagaa	tgtgtccaaac	atcctccggc	ttaaagagag	cattgctgca	480
accaatgaag	ctgtgcacga	ggtaactgac	ggattatcac	aacttagcagt	ggcagtaggg	540
aagatgcaac	agtttgtaa	tgaccagttc	aataatacag	cgcaagaatt	ggactgtata	600
aaaattgcac	agcaggtcgg	tgtagaactc	aacttgtacc	taactgaatt	gactacagta	660
tttggccac	aaatcacttc	ccctgcctta	actcagctga	ctatccaagc	gctttacaat	720
ctagctggtg	gtaatatgga	ttacttgctg	actaagttag	gtgttagggaa	caaccaactc	780
agctcattaa	ttggtagcgg	cttgatcacc	ggcaacccta	ttctgtacga	ctcacagact	840
cagatcttgg	gtatacaggt	aactttgcct	tcaagttggga	acctgaataa	tatgegtgcc	900
acctacctgg	agaccttatac	tgtaagcaca	accaaggat	ttgcctcagc	acttgtccca	960
aaagtggtga	cacaggtcgg	ttccgtgata	gaagaacttg	acacctcata	ctgtataggg	1020

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accgacttgg atttatactg tacaagaata gtgacattcc ctagtctcc tggtatttat    1080
tcttgctga gcggttaatac atcggttgc atgtattcaa agactgaagg cgcaacttact    1140
acgccccata tggctctcaa aggctcagtt attgccaatt gcaagctgac aacatgtaga    1200
tgtgcagatc ccccaggtat catatcgaa aattatggag aagctgtgac cttaatagat    1260
aggcactcat gcaacgtctt atccttagac gggataactc tgaggctcag tggggaaattt    1320
gatgcaacct atcaaaagaa tatctctata ctagattctc aagttatagt gacaggcaat    1380
cttgatatat caactgagct tgggaatgtc aacaactcaa taagtaatgc cctgaataag    1440
tttagaggaaa gcaacagcaa actagacaaa gtcaatgtca aactgaccag cacatctgct    1500
ctcattacct acatcgaaaa aactgtcata tctcttgaaa ttgggtgact tagcctgggt    1560
ctagcatgct acctgtatgtaa caagaaaaaa gcacaacaaa agaccctgtt atggcttggg    1620
aataataaccc ttgatcagat gagagccact aaaaaaatat ga                                1662

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<210> SEQ ID NO 35
<211> LENGTH: 553
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: NDV-F protein from wildtype YZCQ strain
(Amino Acid Sequence of NDV-F of Texas strain with lentogenic
cleavage site sequence)

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<400> SEQUENCE: 35

Met	Gly	Ser	Arg	Ser	Ser	Thr	Arg	Ile	Pro	Val	Pro	Leu	Met	Leu	Ile
1								5		10			15		

Ile	Arg	Thr	Ala	Leu	Thr	Leu	Ser	Cys	Ile	Arg	Leu	Thr	Ser	Ser	Leu
	20							25				30			

Asp	Gly	Arg	Pro	Leu	Ala	Ala	Gly	Ile	Val	Val	Thr	Gly	Asp	Lys
	35						40				45			

Ala	Val	Asn	Ile	Tyr	Thr	Ser	Ser	Gln	Thr	Gly	Ser	Ile	Ile	Val	Lys
	50					55				60					

Leu	Leu	Pro	Asn	Met	Pro	Lys	Asp	Lys	Glu	Val	Cys	Ala	Lys	Ala	Pro
65					70				75			80			

Leu	Glu	Ala	Tyr	Asn	Arg	Thr	Leu	Thr	Thr	Leu	Leu	Thr	Pro	Leu	Gly
	85						90					95			

Asp	Ser	Ile	Arg	Arg	Ile	Gln	Glu	Ser	Val	Thr	Thr	Ser	Gly	Gly
	100					105				110				

Lys	Gln	Gly	Arg	Leu	Ile	Gly	Ala	Ile	Ile	Gly	Ser	Val	Ala	Leu	Gly
	115				120				125						

Val	Ala	Thr	Ala	Ala	Gln	Ile	Thr	Ala	Ala	Ser	Ala	Leu	Ile	Gln	Ala
130					135				140						

Asn	Gln	Asn	Ala	Ala	Asn	Ile	Leu	Arg	Leu	Lys	Glu	Ser	Ile	Ala	Ala
145						150			155			160			

Thr	Asn	Glu	Ala	Val	His	Glu	Val	Thr	Asp	Gly	Leu	Ser	Gln	Leu	Ala
	165					170			175						

Val	Ala	Val	Gly	Lys	Met	Gln	Gln	Phe	Val	Asn	Asp	Gln	Phe	Asn	Asn
	180				185				190						

Thr	Ala	Gln	Glu	Leu	Asp	Cys	Ile	Lys	Ile	Ala	Gln	Gln	Val	Gly	Val
	195				200			205							

Glu	Leu	Asn	Leu	Tyr	Leu	Thr	Glu	Leu	Thr	Thr	Val	Phe	Gly	Pro	Gln
210					215				220						

Ile	Thr	Ser	Pro	Ala	Leu	Thr	Gln	Leu	Thr	Ile	Gln	Ala	Leu	Tyr	Asn
225					230				235			240			

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Leu Ala Gly Gly Asn Met Asp Tyr Leu Leu Thr Lys Leu Gly Val Gly
 245 250 255
 Asn Asn Gln Leu Ser Ser Leu Ile Gly Ser Gly Leu Ile Thr Gly Asn
 260 265 270
 Pro Ile Leu Tyr Asp Ser Gln Thr Gln Ile Leu Gly Ile Gln Val Thr
 275 280 285
 Leu Pro Ser Val Gly Asn Leu Asn Asn Met Arg Ala Thr Tyr Leu Glu
 290 295 300
 Thr Leu Ser Val Ser Thr Thr Lys Gly Phe Ala Ser Ala Leu Val Pro
 305 310 315 320
 Lys Val Val Thr Gln Val Gly Ser Val Ile Glu Glu Leu Asp Thr Ser
 325 330 335
 Tyr Cys Ile Gly Thr Asp Leu Asp Leu Tyr Cys Thr Arg Ile Val Thr
 340 345 350
 Phe Pro Met Ser Pro Gly Ile Tyr Ser Cys Leu Ser Gly Asn Thr Ser
 355 360 365
 Ala Cys Met Tyr Ser Lys Thr Glu Gly Ala Leu Thr Thr Pro Tyr Met
 370 375 380
 Ala Leu Lys Gly Ser Val Ile Ala Asn Cys Lys Leu Thr Thr Cys Arg
 385 390 395 400
 Cys Ala Asp Pro Pro Gly Ile Ile Ser Gln Asn Tyr Gly Glu Ala Val
 405 410 415
 Ser Leu Ile Asp Arg His Ser Cys Asn Val Leu Ser Leu Asp Gly Ile
 420 425 430
 Thr Leu Arg Leu Ser Gly Glu Phe Asp Ala Thr Tyr Gln Lys Asn Ile
 435 440 445
 Ser Ile Leu Asp Ser Gln Val Ile Val Thr Gly Asn Leu Asp Ile Ser
 450 455 460
 Thr Glu Leu Gly Asn Val Asn Asn Ser Ile Ser Asn Ala Leu Asn Lys
 465 470 475 480
 Leu Glu Glu Ser Asn Ser Lys Leu Asp Lys Val Asn Val Lys Leu Thr
 485 490 495
 Ser Thr Ser Ala Leu Ile Thr Tyr Ile Val Leu Thr Val Ile Ser Leu
 500 505 510
 Val Phe Gly Val Leu Ser Leu Val Leu Ala Cys Tyr Leu Met Tyr Lys
 515 520 525
 Gln Lys Ala Gln Gln Lys Thr Leu Leu Trp Leu Gly Asn Asn Thr Leu
 530 535 540
 Asp Gln Met Arg Ala Thr Thr Lys Ile
 545 550

<210> SEQ_ID NO 36
 <211> LENGTH: 1662
 <212> TYPE: DNA
 <213> ORGANISM: artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: NDV-F Texas wildtype DNA sequence

<400> SEQUENCE: 36

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ctgtatattgg actgtatccg tccgacaagc tctcttgacg gcaggcctct tgcagctgca	120
ggaatttgtag taacaggaga taaggcagtc aatgttatata cctcgctcga gacagggtca	180
atcatagtc aagtgtccc gaatatgccca aaggataagg aggcgtgtgc gaaagaccca	240

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ttagaggcat ataacagaac actgactact ttgtcactc ctctggcga atccatccgc 300
aagatccaag ggtctgtgtc cacgtctgga ggaggcaagc aaggccgcct gataggtgt 360
gttattggta gtgttagctct tggggttgca acagcgccac aaataacagc agctcgccgc 420
ctaatacacaag ccaaccagaa tgctgccaac atcccttcggc ttaaggagag cattgtgca 480
accaatgaag ctgtgcatga agtcaccgac ggattatcac aactatcagt ggcagttggg 540
aagatgcagc agtttgtcaa tgaccagttt aataatacag cgcgagaattt ggactgtata 600
aaaatcacac aacaggttgg ttagaactc aacctatacc taactgaattt gactacagta 660
ttcggggccac agatcacccctc ccctgcatta actcagctga ccatccaggc actttataat 720
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ctcattacctt atattgttctt gactgtcatt tctcttagttt tcgggtcact aagttctgggt 1560
ttaacatgtt acctgtatgtt caaacaaaaag gcacaacaaa agaccttgctt atggcttggg 1620
aataataccccc tcqatcqat qaqadccact acaaqagcat qa 1662

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<210> SEQ ID NO 37
<211> LENGTH: 553
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: NDV-F protein from wildtype Texas strain
      (Amino Acid Sequence of NDV-F VIId wt YZCQ with lentogenic
      cleavage site sequence)
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<400> SEQUENCE: 37

Met Gly Ser Lys Pro Ser Thr Arg Ile Pro Ala Pro Leu Met Leu Ile
1 5 10 15

Thr Arg Ile Met Leu Ile Leu Asp Cys Ile Arg Pro Thr Ser Ser Leu
20 25 30

Asp Gly Arg Pro Leu Ala Ala Ala Gly Ile Val Val Thr Gly Asp Lys
35 40 45

Ala Val Asn Val Tyr Thr Ser Ser Gln Thr Gly Ser Ile Ile Val Lys
50 55 60

Leu	Leu	Pro	Asn	Met	Pro	Lys	Asp	Lys	Glu	Ala	Cys	Ala	Lys	Asp	Pro
65					70				75						80

Leu Glu Ala Tyr Asn Arg Thr Leu Thr Thr Leu Leu Thr Pro Leu Gly
85 90 95

Glu Ser Ile Arg Lys Ile Gln Gly Ser Val Ser Thr Ser Gly Gly Gly

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148

100	105	110
Lys Gln Gly Arg Leu Ile Gly Ala Val Ile Gly Ser Val Ala Leu Gly		
115	120	125
Val Ala Thr Ala Ala Gln Ile Thr Ala Ala Ala Leu Ile Gln Ala		
130	135	140
Asn Gln Asn Ala Ala Asn Ile Leu Arg Leu Lys Glu Ser Ile Ala Ala		
145	150	155
Thr Asn Glu Ala Val His Glu Val Thr Asp Gly Leu Ser Gln Leu Ser		
165	170	175
Val Ala Val Gly Lys Met Gln Gln Phe Val Asn Asp Gln Phe Asn Asn		
180	185	190
Thr Ala Arg Glu Leu Asp Cys Ile Lys Ile Thr Gln Gln Val Gly Val		
195	200	205
Glu Leu Asn Leu Tyr Leu Thr Glu Leu Thr Thr Val Phe Gly Pro Gln		
210	215	220
Ile Thr Ser Pro Ala Leu Thr Gln Leu Thr Ile Gln Ala Leu Tyr Asn		
225	230	235
Leu Ala Gly Gly Asn Met Asp Tyr Leu Leu Thr Lys Leu Gly Ile Gly		
245	250	255
Asn Asn Gln Leu Ser Ser Leu Ile Gly Ser Gly Leu Ile Thr Gly Tyr		
260	265	270
Pro Ile Leu Tyr Asp Ser Gln Thr Gln Leu Leu Gly Ile Gln Val Asn		
275	280	285
Leu Pro Ser Val Gly Asn Leu Asn Asn Met Arg Ala Thr Tyr Leu Glu		
290	295	300
Thr Leu Ser Val Ser Thr Ala Lys Gly Tyr Ala Ser Ala Leu Val Pro		
305	310	315
Lys Val Val Thr Gln Val Gly Ser Val Ile Glu Glu Leu Asp Thr Ser		
325	330	335
Tyr Cys Ile Glu Ser Asp Leu Asp Leu Tyr Cys Thr Arg Ile Val Thr		
340	345	350
Phe Pro Met Ser Pro Gly Ile Tyr Ser Cys Leu Ser Gly Asn Thr Ser		
355	360	365
Ala Cys Met Tyr Ser Lys Thr Glu Gly Ala Leu Thr Thr Pro Tyr Met		
370	375	380
Ala Leu Lys Gly Ser Val Ile Ala Asn Cys Lys Ile Thr Thr Cys Arg		
385	390	395
Cys Thr Asp Pro Pro Gly Ile Ile Ser Gln Asn Tyr Gly Glu Ala Val		
405	410	415
Ser Leu Ile Asp Arg His Ser Cys Asn Val Leu Ser Leu Asp Gly Ile		
420	425	430
Thr Leu Arg Leu Ser Gly Glu Phe Asp Ala Thr Tyr Gln Lys Asn Ile		
435	440	445
Ser Ile Leu Asp Ser Gln Val Ile Val Thr Gly Asn Leu Asp Ile Ser		
450	455	460
Thr Glu Leu Gly Asn Val Asn Asn Ser Ile Ser Asn Ala Leu Asp Lys		
465	470	475
Leu Ala Lys Ser Asn Ser Lys Leu Glu Lys Val Asn Val Arg Leu Thr		
485	490	495
Ser Thr Ser Ala Leu Ile Thr Tyr Ile Val Leu Thr Val Ile Ser Leu		
500	505	510
Val Phe Gly Ala Leu Ser Leu Gly Leu Thr Cys Tyr Leu Met Tyr Lys		
515	520	525

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Gln Lys Ala Gln Gln Lys Thr Leu Leu Trp Leu Gly Asn Asn Thr Leu
 530 535 540

Asp Gln Met Arg Ala Thr Thr Arg Ala
 545 550

<210> SEQ ID NO 38
 <211> LENGTH: 622
 <212> TYPE: DNA
 <213> ORGANISM: artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: MDV gB promoter
 <400> SEQUENCE: 38

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atagcagtag aaaaacagat tggaccgtca gtaagtttag agggtttat gacttagca   180
ctatagataa tgtaactgcg gcccatcgca tggcttggaa atatatcaa gaactgattt   240
ttgcaacagc ttatatttct tctgtattta aatgtggcga attgcacatc tgcgtgccg   300
acagtttgca gatcaacagc aatggagact atgtatggaa aaatggata tatataacat   360
atgaaaccga atatccactt ataatgattc tggggtcaga atcaagoact tcagaaacgc   420
aaaaatgac tgcaattatt gatacagatg tttttcggtt gctttatct attttgcagt   480
atatggcccc cggtacggca gatcagggtc gagtagaaca gattaccaac agccacgccc   540
ccatctgacc cgtccaatat tcttgtgtcc ctgcattttt tctcacacaa tttatgaaca   600
gcacattaa gatcatctca ct   622
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<210> SEQ ID NO 39
 <211> LENGTH: 4850
 <212> TYPE: DNA
 <213> ORGANISM: artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Partial plasmid SORF3-US2 gpVar-Ewtsyn sequence
 for vHVT202
 <400> SEQUENCE: 39

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cattatttgg atatcttccg gttgtcccat atcccgccct ggtaccgctc ggataccctg   180
cccgatggaa ttctgttgcgca cagtcgcgcga atcggggacc aacaacgcgt gggtccacac   240
tcatttcggaa atttcccgat gattctgaat atttattgcc gtcgttacg agtcgttggaa   300
catatctgtta atacatttct tcttcgttgcgca catttgcgtt atacatttgcgca   360
caggatgttc aagtctcaga tggcacatc tggcacagca caactttatg gcatttcgaa   420
tgtaatcgatc cggcagccct gggggagttc tatattcgca tattggatg gtaaggacaa   480
tagcagatct cgcaacctcc agggaggcta taataacgtt tttaaaggat ggatttcgtca   540
taaaaatctg tcgcaaaatcactgagaat atcctttact agcgccgatt gagagcatcg   600
tcgtccaaatt ttctaaatgg aaagaaaaca aggccccaa gagtgttcca aacatttca   660
tttcggcga atctctcaaa tcccatggcg tgcaattgtat tgcaaaatgg gcacttcgt   720
tcacgttgcgtt atctccaaac tctaaagacac tttaattgtaa aaaactacgt tctgttgg   780
aaagaaaacct ataggcagac catagaacta tttgacacca catatcttt tggatgtcaa   840
actgaccatc atcgatgtt gctgaatgccttgcgtccatc gactccatc   900
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attgaataat tccacacgtc agctcatcg tttagcaagg ccagtagttg aagtcattt 960
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 caccggctct ggtcatggtg aaggaacttg tagcataaaag acgcaggtat catagggta 1080
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 cctgcagggtt agtcatatgt tacttggcag aggccgcgtg gaaagtcct ggacgtggg 1260
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gactttcgta agtacttcata ggaggtggcc gacctaact ctccccctgaa gattgcagga	3420
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cgtatggaaatc agatgttgag tcttcgaaag taatgtcctc gaatatgggt attgtctgt	4740
aaaaatatcga aagcggtaacg acggttgcag aaccgtcgat gtcgcccagat actagtaaca	4800
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<210> SEQ_ID NO 40
 <211> LENGTH: 4943
 <212> TYPE: DNA
 <213> ORGANISM: artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Partial plasmid SB1US2 gpVIIIdwtsyn sequence for
 vSB1-010

<400> SEQUENCE: 40	
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gttggcgagg tgccttcgt gactctgaaat tggcccttag cggcttccac tatctcgta	180
aaggctctat aatacagttc ctctgcagac ccgtcggtgc tcttccttc tgcgtcgta	240
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tccctatct cgtctccgg taatcccata ggtgttcggg attcgcagat aggttagagaa	360
agcaccactg caaatcgta aattccatt gccccaaacca atatTTTT taagaacggc	420

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gtgtgaatcg atagactaa catacgctctt ccataaaacaaaacaaaacaaaact	3780
agcaaaaatag gctgtccccca gtgcaagtgc aggtgccaga acatttctct tctagacctg	3840
caggggggcgtc tgcgtcaaggtaatgaccctt cgccgttcat tcggaaagttta taactgcgc	3900
cttcgeacat ttcttttgcgtt cctgtttgtt attgccataa cagataggaa ttgaaacactg	3960
atcctctctgtt ttttgcgtc atggccagca acagaataact ttgtcgatgc gactactgc	4020
gctggatgggtt tccgttcttgcgtt gagggttcgg cgggtcggtt ggagaaccta ttatgttata	4080
cacacacgtc ataccgttgcgtt ccgcggaaatgc ttctttgtct tctgcgtctt cgaaacgtcg	4140
ttccccacgtt gacgttagga gctgttggaaat ggtatcgatggaaagccacgc gcatgcggaa	4200
ccaaatgttgcgtt gctactttgtt ccgcggacgcgt tctcttcggat aatggggatgtt attccagagc	4260
agcgcggcgcg agatcagcgcccccactat ccacagactgtatgttgcgtt tttctgaaac	4320
atcggactcc aacatcaaataat atccagacat aacatcttgc cattcgaaag cacatccgc	4380
gacatcttca aatagcctaa ctataaaacgtt gtctcttagttt cctgctaaacc cagtagctcg	4440
aatggccgtc ccataccgtt ggttcgttccgtt gataatcggtt ctctgacgc gaggaaac	4500
taaaagggtt ctggaaaagc ggaacagatc tgcagaccga acgactacag acacgcccac	4560
atcatcatgtt atctgttccat tgcattgtttt tatgagaaaa atccataagg ccgaggccgc	4620
atctctatgtt ctccggggat ttctctcgat ctcattcttgcgtt agagtgcgtt cagttatcat	4680
agacacgccccccat ttttgcgttccgtt cttccgttac tgggtggagcg tcggccggg	4740
aatcggtccgtt tgctctgtttt ccagtgttcaat gacagaagac catccgtttaa attctgggtgtt	4800
atgaactgac ggtctccaga cgaacgtcgat gacatggaaactt aacgagcttt	4860
cttcggaaatgtt gtctgttccgtt aacgcttcaat gacatggaaactt aacgagcttt	4920
gtgacacaga tccgtcggtt tcgtt	4943

<210> SEQ ID NO 41

<211> LENGTH: 1362

<212> TYPE: DNA

<213> ORGANISM: artificial sequence

<220> FEATURE:

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-continued

<223> OTHER INFORMATION: IBDV DNA encoding VP2 protein of IBDV E strain

<400> SEQUENCE: 41

atgacaacaaacc tgcaagatca aacccaaacag attgttccgt tcatacggag cctttgtatg
ccaacaacccg gaccggcgtc cattccggac gacacctgg agaagcacac ttcagggtca
gagacacctga cctacaattt gactgtgggg gacacagggt cagggtaat tgttttttc
cctggattcc ctggctaat tgtgggtgt cactacacac tgcagagcaa tgggaaactac
aagtgcatac agatgtcct gactgccag aacotacccg ccagctacaa ctactgcagg
ctagttagtc ggagtctcac agtaagggtca agcacactcc ctgggtgggt ttatgcacta
aacggcacca taaacgcgt gacccctaa ggaaggctga gtgaactgac agatgttagc
tacaacccggg tcatgtctgc aacagccaa acatcaacgaca aaattggaa cgtccctagta
ggggaaagggg taaccgtcct cagcttaccc acatcatatg atcttggta tgtgaggctt
ggtgacccca taccgcata agggcttgac cccaaaatgg tagcaacatg tgacagcagt
gacaggccca gagtctacac cataactgca gcccataatt accaattctc atcacagtag
caaacaggtg gggtaacaat cacactgttc tcagccaaaca ttgtatccat cacaagtctc
agcgttgggg gagagcttgt gttcaaaaca agcgccaaac gccttgcact gggccacc
atctacccat taggcttgc tggactgca gtaatcacca gagctgtggc cgccaaacaat
gggctgacgg cccgcatacga caatcttgcg ccattcaatc ttgtgattcc aaccaatgag
ataacccacg caatcacatc catcaaactg gagatagtga cctccaaag tcatgttgc
gcaggggaaac agatgtcatg gtcggcaagt gggagcttag cagtgacgat ccatgggtgc
aactatccag gagccctccg tcccgatcaca ctatgtgcgt acgaaagagt ggcaacagga
tctgtcgatc cggatcgatgg ggtgatcacaatc ttgcagatgc tcccaatcc tgaactagca
aagaacccgg ttacagaata tggccgatatt gacccaggag ccatgaacta cacgaaattg
atactgatgt agagggacccg ccttggatc aagaccgtt ggcacaaacag ggatgtacact
gactttcgatc agtacttcat ggagggtggcc gacccatctt cttccctgaa gattgcagg
qcattttqct tcaaaqacat aatccqqqccq ataaqqqaaqt qa
1362

<210> SEQ ID NO 42

<211> LENGTH: 453

<212> TYPE: PRT

<213> ORGANISM: artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: IBDV VP2 protein of IBDV E strain

<400> SEQUENCE: 42

Met	Thr	Asn	Leu	Gln	Asp	Gln	Thr	Gln	Gln	Ile	Val	Pro	Phe	Ile	Arg
1				5				10					15		

Ser Leu Leu Met Pro Thr Thr Gly Pro Ala Ser Ile Pro Asp Asp Thr
20 25 30

Leu Glu Lys His Thr Leu Arg Ser Glu Thr Ser Thr Tyr Asn Leu Thr
35 40 45

Val Gly Asp Thr Gly Ser Gly Leu Ile Val Phe Phe Pro Gly Phe Pro
50 55 60

65 70 75 80
Lys Phe Asp Gln Met Leu Leu Thr Ala Gln Asn Leu Pro Ala Ser Tyr

Agn Thym Chia Ang Lou Vol. San Ang San Lou Thar Vol. Ang San San Thar

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100	105	110
Leu Pro Gly Gly Val Tyr Ala Leu Asn Gly Thr Ile Asn Ala Val Thr		
115	120	125
Phe Gln Gly Ser Leu Ser Glu Leu Thr Asp Val Ser Tyr Asn Gly Leu		
130	135	140
Met Ser Ala Thr Ala Asn Ile Asn Asp Lys Ile Gly Asn Val Leu Val		
145	150	155
Gly Glu Gly Val Thr Val Leu Ser Leu Pro Thr Ser Tyr Asp Leu Gly		
165	170	175
Tyr Val Arg Leu Gly Asp Pro Ile Pro Ala Ile Gly Leu Asp Pro Lys		
180	185	190
Met Val Ala Thr Cys Asp Ser Ser Asp Arg Pro Arg Val Tyr Thr Ile		
195	200	205
Thr Ala Ala Asp Asn Tyr Gln Phe Ser Ser Gln Tyr Gln Thr Gly Gly		
210	215	220
Val Thr Ile Thr Leu Phe Ser Ala Asn Ile Asp Ala Ile Thr Ser Leu		
225	230	235
Ser Val Gly Gly Glu Leu Val Phe Lys Thr Ser Val Gln Ser Leu Val		
245	250	255
Leu Gly Ala Thr Ile Tyr Leu Ile Gly Phe Asp Gly Thr Ala Val Ile		
260	265	270
Thr Arg Ala Val Ala Ala Asn Asn Gly Leu Thr Ala Gly Ile Asp Asn		
275	280	285
Leu Met Pro Phe Asn Leu Val Ile Pro Thr Asn Glu Ile Thr Gln Pro		
290	295	300
Ile Thr Ser Ile Lys Leu Glu Ile Val Thr Ser Lys Ser Asp Gly Gln		
305	310	315
Ala Gly Glu Gln Met Ser Trp Ser Ala Ser Gly Ser Leu Ala Val Thr		
325	330	335
Ile His Gly Asn Tyr Pro Gly Ala Leu Arg Pro Val Thr Leu Val		
340	345	350
Ala Tyr Glu Arg Val Ala Thr Gly Ser Val Val Thr Val Ala Gly Val		
355	360	365
Ser Asn Phe Glu Leu Ile Pro Asn Pro Glu Leu Ala Lys Asn Leu Val		
370	375	380
Thr Glu Tyr Gly Arg Phe Asp Pro Gly Ala Met Asn Tyr Thr Lys Leu		
385	390	395
Ile Leu Ser Glu Arg Asp Arg Leu Gly Ile Lys Thr Val Trp Pro Thr		
405	410	415
Arg Glu Tyr Thr Asp Phe Arg Glu Tyr Phe Met Glu Val Ala Asp Leu		
420	425	430
Asn Ser Pro Leu Lys Ile Ala Gly Ala Phe Gly Phe Lys Asp Ile Ile		
435	440	445
Arg Ala Ile Arg Arg		
450		

<210> SEQ_ID NO 43
<211> LENGTH: 884
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Guinea pig CMV promoter

<400> SEQUENCE: 43

ttagtcatat gttacttggc agaggccgca tggaaagtcc ctggacgtgg gacatctgat 60

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taataacgtga ggaggtcagc catgttcttt ttggcaaagg actacggtca ttggacgtt	120
gattggcatg ggatagggtc agccagagtt aacagtgttc ttttggcaaagg gggatacgtg	180
gaaaagtcccc ggccatttac agtaaactga tacggggaca aagcacagcc atattnagtc	240
atgttattgtc tggcagaggg tctatggaaa gtccctggac gtgggacgac tgattaatata	300
gaaaagaaggc cagccagagg tagctgtgtc cttttggca aagggatacg gttatggac	360
gtttgattgg actgggatag ggtcagccag agttaacagt gttttttgg caaaggaaac	420
gtggaaagtc ccggggcatt tacagtaaac tgatactggg acaaagtaca cccatattta	480
gtcatgttct ttttggcaaa gagcatctgg aaagtcccg gcaagcattat agtcaacttgg	540
cagaggggaaa gggtaactca gagtttaagta catctttcca gggcaatat tccagtaaat	600
tacacttagt tttatgc当地 tcagccacaa aggggatttt cccggtaat tatgacttt	660
tccttagtca tgcggtatcc aattactgcc aaattggcag tacatacttag gtgattcact	720
gacatttggc cgtcctctgg aaagtccctg gaaaccgctc aagtactgtt tcatgggac	780
tttgcatttt tggagagcac gccccactcc accattggtc cacgtaccct atgggggagt	840
ggtttatgag tatataaggg gctccggttt agaagccggg caga	884

<210> SEQ ID NO 44
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer HM101

<400> SEQUENCE: 44

cggaaattcc gatgtttagt cacgatagac	30
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<210> SEQ ID NO 45
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer HM102

<400> SEQUENCE: 45

ataagagccg ccgcagttagt atgatcttaa tgatg	35
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<210> SEQ ID NO 46
<211> LENGTH: 38
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer F-ATG

<400> SEQUENCE: 46

tatagcggcc gcaagatggg ctccagatct tctaccag	38
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<210> SEQ ID NO 47
<211> LENGTH: 34
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer F-STOP

<400> SEQUENCE: 47

cggggggcc gctcatatcc ttgttagtggc tctc	34
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What we claim is:

1. A composition or vaccine comprising one recombinant herpesvirus of turkeys (HVT) vector comprising at least one or more heterologous polynucleotides coding for and expressing at least one antigen of an avian pathogen, wherein the polynucleotide encodes a Newcastle Disease Virus F (NDV-F) polypeptide and is operably linked to an SV40 promoter, and wherein the polynucleotide is codon-optimized.

2. The composition or vaccine of claim 1, wherein the NDV-F polypeptide has at least 95% sequence identity to the amino acid sequence as set forth in SEQ ID NO:2, 4, 6, 33, 35, or 37.

3. The composition or vaccine of claim 1, wherein the polynucleotide encoding the NDV-F polypeptide is operably linked to an SV40 polyA signal.

4. The composition or vaccine of claim 3, wherein the polynucleotide encoding the NDV-F polypeptide, the operably linked SV40 promoter, and the SV40 polyA signal are inserted in the IG1 (intergenic region 1) locus of HVT genome.

5. The composition or vaccine of claim 1, wherein the composition or vaccine further comprises a second recombinant HVT vector comprising a heterologous polynucleotide coding for and expressing IBDV VP2 antigen.

6. The composition or vaccine of claim 5, wherein the second recombinant HVT vector is the HVT vector included in VAXXITEK™ HVT+IBD.

7. The composition or vaccine of claim 1 or 5, wherein the composition or vaccine is a multivalent composition further comprising one or more recombinant SB1 vectors or the parental SB1 strain.

8. The composition or vaccine of claim 7, wherein the recombinant SB1 vector comprises one or more heterologous polynucleotides coding for and expressing a Newcastle Disease Virus F (NDV-F) antigen.

9. The composition or vaccine of claim 8, wherein the SB1 vector is selected from the group consisting of an SB1 vector comprising an SV40 promoter and a polynucleotide encoding an NDV-F antigen inserted in the region coding for glycoprotein C (UL44) of the SB1 vector, an SB1 vector comprising a guinea pig CMV promoter and a polynucleotide encoding an NDV-F antigen inserted in the region between SORF4 and US2 of the SB1 vector, an SB1 vector comprising an mCMV IE promoter and a polynucleotide encoding an NDV-F antigen inserted in the region US10 of the SB1 vector, and an SB1 vector comprising an SV40 promoter and a polynucleotide encoding an NDV-F antigen inserted in the region between UL55 and LORF5 of the SB1 vector.

10. The composition or vaccine of claim 1, wherein the HVT vector comprises a first heterologous polynucleotide coding for and expressing a Newcastle Disease Virus F (NDV-F) antigen having at least 95% sequence identity to the amino acid sequence as set forth in SEQ ID NO:2, 4, 6, 33, 35, or 37 and a second heterologous polynucleotide coding for and expressing an Infectious Bursal Disease Virus (IBDV) VP2 antigen having at least 95% sequence identity to the amino acid sequence as set forth in SEQ ID NO:8 or 42.

11. The composition or vaccine of claim 10, wherein the polynucleotide encoding the IBDV VP2 antigen is operably linked to a CMV promoter.

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12. The composition or vaccine of claim 11, wherein the polynucleotide encoding the NDV-F antigen is operably linked to an SV40 polyA signal and the polynucleotide encoding the IBDV VP2 antigen is operably linked to an SV40 polyA signal.

13. The composition or vaccine of claim 12, wherein the polynucleotide encoding the NDV-F antigen, the operably linked SV40 promoter, and the SV40 polyA signal are inserted in the IG1 locus of the HVT genome, and wherein the polynucleotide encoding the IBDV VP2 antigen, the operably linked CMV promoter, and the SV40 polyA signal are inserted in the IG1 locus or the SORF3-US2 locus of the HVT genome.

14. The composition or vaccine of claim 10, wherein the composition or vaccine further comprises a second recombinant HVT vector comprising a heterologous polynucleotide coding for and expressing an IBDV VP2 antigen.

15. The composition or vaccine of claim 14, wherein the recombinant HVT vector is the HVT vector included in VAXXITEK™ HVT+IBD.

16. The composition or vaccine of claim 10, wherein the composition or vaccine is a multivalent composition or vaccine further comprising one or more recombinant SB1 vectors or the parental SB1 strain.

17. The composition or vaccine of claim 16, wherein the recombinant SB1 vector comprises one or more heterologous polynucleotides coding for and expressing a Newcastle Disease Virus F (NDV-F) antigen.

18. The composition or vaccine of claim 17, wherein the SB1 vector is selected from the group consisting of an SB1 vector comprising an SV40 promoter and a polynucleotide encoding an NDV-F antigen inserted in the region coding for glycoprotein C (UL44) of the SB1 vector, an SB1 vector comprising a guinea pig CMV promoter and a polynucleotide encoding an NDV-F antigen inserted in the region between SORF4 and US2 of the SB1 vector, an SB1 vector comprising an mCMV IE promoter and a polynucleotide encoding an NDV-F antigen inserted in the region US10 of the SB1 vector, and an SB1 vector comprising an SV40 promoter and a polynucleotide encoding an NDV-F antigen inserted in the region between UL55 and LORF5 of the SB1 vector.

19. A method of vaccinating an animal comprising at least one administration of the composition or vaccine of claim 1, wherein the animal is avian.

20. A method for inducing an immunogenic or protective response in an animal against one or more avian pathogens comprising at least one administration of the composition or vaccine of claim 1, wherein the animal is avian.

21. The method of claim 20, wherein the avian pathogen is selected from the group consisting of Newcastle Disease Virus (NDV), Infectious Bursal Disease Virus (i.e., IBDV or Gumboro Disease virus), Marek's Disease Virus (MDV), Infectious Laryngotracheitis Virus (ILTV), avian encephalomyelitis virus, avian reovirus, avian paramyxovirus, avian metapneumovirus, avian influenza virus, avian adenovirus, fowl pox virus, avian coronavirus, avian rotavirus, avian parvovirus, avian astrovirus and chick anemia virus coccidiosis (*Eimeria* sp.), *Campylobacter* sp., *Salmonella* sp., *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, *Pasteurella* sp., *Avibacterium* sp., *E. coli* and *Clostridium* sp.

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